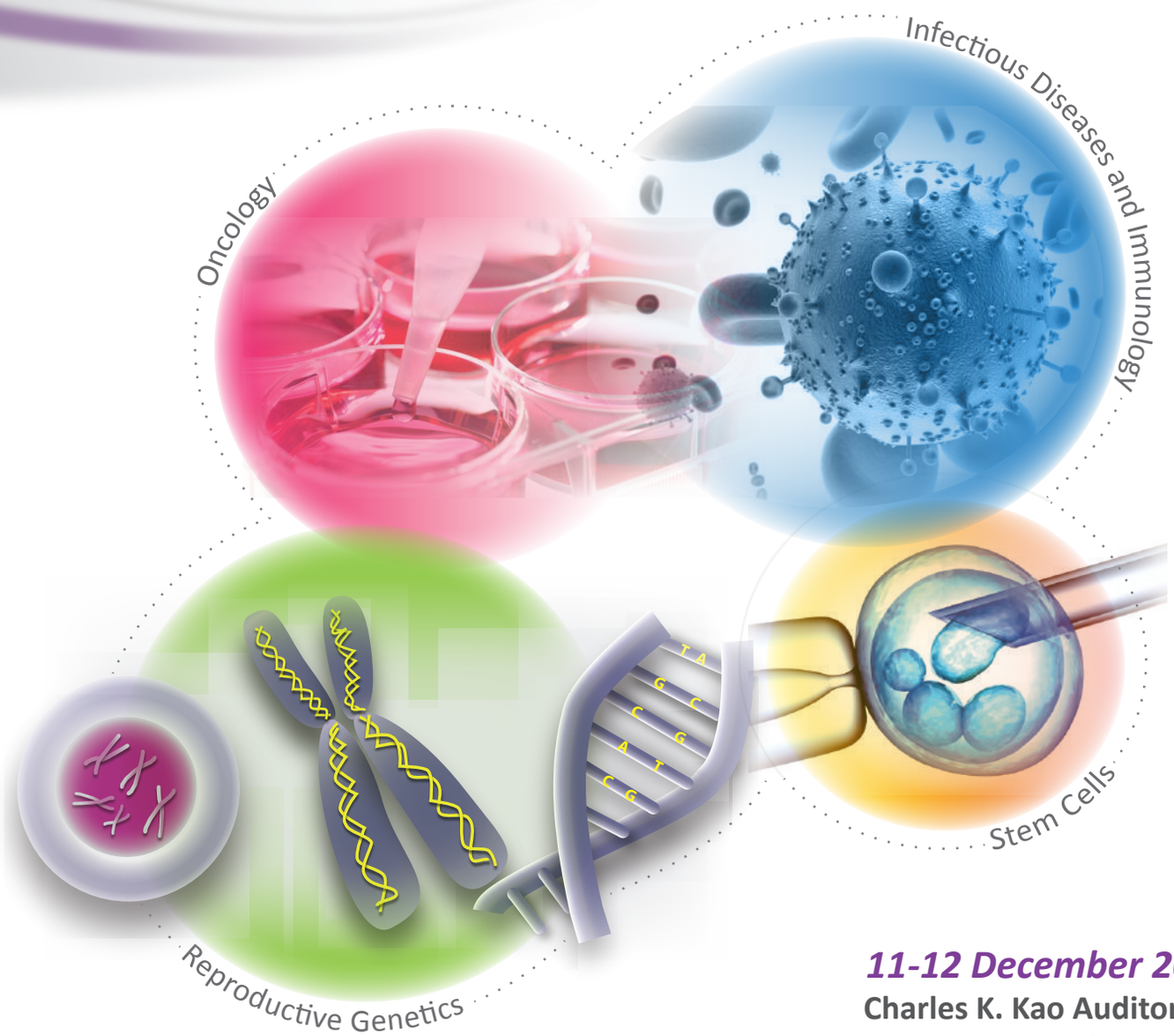
ASIA-International Biomedical Science Consortium

# Pan-Asian Biomedical Sciences Conference




**11-12 December 2014**
**Charles K. Kao Auditorium**
**Hong Kong Science Park**
**Hong Kong**

**Organizer:**

School of Biomedical Sciences
Faculty of Medicine
The Chinese University of Hong Kong
香港中文大學醫學院生物醫學學院

**Co-organizer:**

Hong Kong 香港科技園
Science & Technology Parks

# Pan-Asian Biomedical Sciences Conference

ASIA-International Biomedical Science Consortium

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*Bookcover was designed by Ms. Tai Fung WAN, School of Biomedical Sciences, CUHK*

# Welcome Message from the Director of School of Biomedical Sciences

Pro-Vice Chancellor Prof. Fanny Cheung, Vice President of the Hong Kong Science and Technology Parks Corporation Mr. Andrew Young, honored guests from the Pan-Asian Biomedical Science Consortium, honor guests, colleagues, students, ladies and gentlemen, I am most delighted to welcome you all to the Pan-Asian Biomedical Sciences Conference cum School of Biomedical Sciences Postgraduate Research Day 2014.

This Conference is the 2nd conference organized by the ASIA-International Biomedical Science Consortium, comprising 12 universities from 4 different countries. The Consortium aims to promote biomedical sciences research and also collaborations in postgraduate education in the Pan-Asian Region. It is our honor to host this 2nd Pan-Asian Biomedical Sciences Conference in Hong Kong. I would like to extend the warmest welcome to all the speakers, guests, members of the Consortium and their graduate students for joining this flagship event.

This year, the Conference focuses in four research areas namely, Infectious Diseases & Immunology, Oncology, Reproductive Genetics and Stem Cells, respectively, with more than 20 invited speakers from member institutions of the Consortium and Hong Kong. I would also like to proudly present to you our Keynote Speakers who are experts in the above four main areas: they are Professor Chang Tse-wen, Academia Sinica of Taiwan, Professor Dennis Lo, The Chinese University of Hong Kong, Professor Suh Yoo-hun, Korea Brain Research Institute, and Professor Yuen Kwok-yung, The University of Hong Kong. I am sure you will enjoy the program we have prepared for you these two days and find the speeches informative, stimulating, and exciting.

Apart from the Conference, the School of Biomedical Sciences Postgraduate Research Day 2014, which is one of the annual events of our School, will be held in conjunction with the Conference. The Postgraduate Research Day is an event organized by and for our graduate students. This is the 5th Postgraduate Research Day held by our students. Research postgraduate students from our School will share the results of their research through posters and oral presentations. I invite you all to visit the posters and give our students your most valuable comments and suggestions.

Finally, may I take this opportunity to thank the Hong Kong Science and Technology Park, our co-organizer, for providing the state-of-the-art venue, and all the thoughtful planning and support throughout the preparation period. I would also like to thank the members of the Organizing Committee of the Conference, in particular its Chair, Professor Chi–hin Cho, whose earnest efforts and dedication make this event a success.

It is our hope that with the increasing interactions and scientific exchanges amongst investigators in the Pan-Asian Region, our research capacity and strength will grow together and stay competitive with scientists in the other parts of the world. I welcome you once again to the Conference. Besides attending the Conference, I do encourage you to find some time to visit Hong Kong.

Wai Yee Chan, Ph.D.
Professor of Biomedical Sciences
Director, School of Biomedical Sciences
The Chinese University of Hong Kong

## Welcome Message from Pro-Vice-Chancellor, The Chinese University of Hong Kong

Mr. Andrew Young, Vice President of the Hong Kong Science and Technology Parks Corporation and Prof. Wai Yee Chan, the Director of the School of Biomedical Sciences, The Chinese University of Hong Kong, ladies and gentlemen,

On behalf of The Chinese University of Hong Kong (CUHK), it is my great pleasure to welcome you to the Pan-Asian Biomedical Sciences Conference. Biomedical Sciences is one of the five focused areas of research in CUHK. By combining the four former preclinical departments of the Faculty of Medicine, namely Departments of Anatomy, Biochemistry, Pharmacology and Physiology, the School of Biomedical Sciences (the School) was established in 2009. During these five years, the School continuously shows remarkable and encouraging achievements, especially in research outputs and academic outreach. By initiating collaboration with institutions worldwide, the School is expanding its academic network in promoting excellence in the Biomedical Sciences research.

The Pan-Asian Biomedical Sciences Conference, with the involvement of representatives from members of ASIA-International Biomedical Science Consortium and speakers from other renowned institutions, provides us an excellent platform to share with one another their research directions, latest findings and most up-to-date research outcomes in the Pan-Asian Region. With strengthened links among researchers, it is expected that the research capacity of all participants could be enhanced, followed by the potential international recognition and reputation.

With the postgraduate students' participation in the Conference, our future scientists are provided excellent opportunities to showcase their research findings. Through the exchanges with local and overseas fellow students as well as with professional scientists, their potential academic connections with different institutions in the Pan-Asian Region will be established.

Building up strong academic communications and collaborations among institutions and investigators within the region will broaden the research spectrum of individual institutions and enhance the international competitiveness of our Consortium members. International and interdisciplinary research collaborations constitute the key strategic direction of our University. As the Pro-Vice-Chancellor in research of CUHK, I fully support these initiatives of the School of Biomedical Sciences.

Last but not least, I wish all our honorable guests a pleasant stay in Hong Kong and hope you will all enjoy a fruitful meeting.

Prof. Fanny M.C. Cheung
Pro-Vice-Chancellor / Vice-President
The Chinese University of Hong Kong

# Welcome Message from Vice President of Hong Kong Science and Technology Parks Corporation

It is my honor to welcome all of you to the Hong Kong Science Park for the Pan-Asian Biomedical Sciences Conference.

The Hong Kong Science and Technology Parks Corporation is a statutory body set up by the HKSAR Government in 2001. Our missions is to provide a world class platform and value added services to connect stakeholders, nurture talents, and facilitate collaboration that will accelerate innovation and technological advancement that brings value to Hong Kong and the region. Biomedical science is a most important driving force in enriching the quality of life for mankind.

As the co-organizer of the Conference, we are delighted to have your participation in this event to share with each other the latest biomedical sciences research findings in the Pan-Asian Region.

Through the talks hosted by various notable scientists and presentations by outstanding postgraduate students on research directions and results, I believe this conference will be a great opportunity for all of us to share knowledge, generate fascinating discoveries and gain inspiration on how we can expedite the development for biomedical science.

Last but not least, it is our pleasure to co-organize this Conference with the School of Biomedical Sciences from The Chinese University of Hong Kong. I would like to extend my sincere appreciation to the organizing committee and helpers for all their hard work in the preparation and coordination of this important event.

I sincerely wish that you will make the most out of this Conference and have a pleasant stay in Hong Kong.

Andrew Young
Vice President
Hong Kong Science and Technology Parks Corporation

# Biographies of Keynote Speakers

**Tse Wen CHANG,** Ph.D.

Dr. Chang is a Distinguished Research Fellow in the Genomics Research Center, Academia Sinica in Taipei. He received B.S. and M.S. degrees from Tsing Hua University in Taiwan and PhD from Harvard University and did postdoctoral research in M.I.T. After a 4-year stint at Centocor, Dr. Chang was recruited by Baylor College of Medicine as a full Professor in1985. He cofounded Tanox in Houston, Texas in 1986. In 1987, he invented the anti-IgE therapy, leading to the development of omalizumab (Xolair), which has been approved in more than 90 countries for treating severe allergic asthma. Xolair has also been approved in EU, U.S.A., and about 10 other countries in 2014 for treating severe chronic spontaneous urticaria untreatable with standard medicine. In 1990, he invented the "migis" ("membrane-bound immunoglobulin isotype-specific") approach for isotype-specific targeting of B lymphocytes, which has been employed to develop an antibody for the treatment of IgA nephropathy. He also discovered in 1990 a 52-residue domain called CεmX on human membrane-bound IgE. Favorable results have been obtained from Phase II trials on the anti-CεmX antibody, quilizumab. Dr. Chang invented "antibody matrix", a microarray prototype in 1982 and single B cell PCR for preparing human antibodies in 1993. Dr. Chang received an Honorary Fellow Award from American College of Allergy Asthma and Immunology in 2004 and an Honorary Fellow Award from American Academy of Allergy Asthma and Immunology in 2007.

**Yuk Ming Dennis LO,** MA DM DPhil FRCP FRCPath FRS

Professor Lo is the Director of the Li Ka Shing Institute of Health Sciences and the Chairman of the Department of Chemical Pathology of The Chinese University of Hong Kong. In 1997, Prof. Lo and his co-workers reported the presence of cell-free fetal DNA in the plasma of pregnant women. Since then, Prof. Lo has elucidated the fundamental biological characteristics regarding circulating fetal DNA as well as its clinical applications in noninvasive prenatal diagnosis. In 2008, Prof. Lo and his team demonstrated that next-generation sequencing of maternal plasma DNA would allow fetuses with Down syndrome to be detected robustly and noninvasively. In 2011, Prof. Lo and his team published the first large-scale validation of this technology for Down syndrome detection. This technology has since then been rapidly introduced into clinical practice in late 2011. Prof. Lo was also the first to demonstrate in 2010 that the fetal genome could be sequenced noninvasively from maternal plasma. Taken as a whole, Prof. Lo's work has created a paradigm shift in prenatal diagnosis, making such testing safer for the fetuses and less stressful for the pregnant mothers. In recognition of his work, Dr. Lo has won numerous awards and was elected to the Royal Society in 2011, elected as a Foreign Associate of the US National Academy of Sciences in 2013, and won the King Faisal International Prize in Medicine in 2014.

# Biographies of Keynote Speakers

**Yoo Hun SUH**, MD, PhD

Professor Suh is a Korean neuroscientist. He is the president of Korea Brain Research Institute and has demonstrated his ability to promote Korea Brain Research Institute as the foundation for neuroscientists. Furthermore, he is a professor emeritus of College of Medicine at Seoul National University. He won Korea's Most Distinguished Scientist Award, the National Government Medals and many other prizes. He was selected one of 20 outstanding Korean Medical Scientists and one of 21 outstanding Korean Scholars of the 21st Century. He is an editor and editorial board member for 6 SCIs (Journal of Pharmacological Sciences, Journal of Neurochemistry, Journal of Molecular Neuroscience, Journal of Neuroscience Research, Neurochemical Research and Neuroscience Research). He first cloned the gene for epinephrine synthesizing enzyme, PNMT and has greatly contributed to the discovery of a new potential gene and factors for Alzheimer's Disease (AD), the development of potential stem cell and drugs for AD and Parkinson's (PD). He has published more than 200 papers and over 50 books.

Presently, Professor Suh is a Member of Board of Trustee, HFSP (Human Frontier Science Program), a member of Korean National Academy of Science and Technology, a member of Korean National Academy of Medicine and a Member of Korean National Science and Technology council. In addition, he is a Member of International Scientific Advisory Board of AD & PD and a Member of International Scientific Advisory Board of International Conference of Alzheimer's Disease and related disorders (ICAD). He was the chairman of the Organizing Committee of the 22nd Biennial Meeting of International Society of Neurochemistry (ISN), the presidents of FAONS (Federation of Asia, Oceanic Neuroscience Societies), APSN (Asia Pacific Society for Neurochemistry), Korean Society for pharmacology, Korean Society for Brain & Neuroscience, Korean Society for Cognitive Science, Korean Society for Neurodegenerative Diseases and Korean Mind, Brain and Education Society as well as the Directors of National Creative Research Initiatives Center for AD, Neuroscience Research Institute, Seoul National University and Cognitive Science Institute, Seoul National University.

**Kwok Yung YUEN,** MBBS(HK), MD(HK), FRCS(Glas), FRCPath(UK), FRCP(Edin, Lond & Irel)

Professor Yuen is Academician of the Chinese Academy of Engineering (Basic Medicine and Health) and Silver Bauhinea Star Awardee of the Hong Kong Special Administrative Region of China. He is also Fellow of the Royal College of Physicians (Lond, Edin), Surgeons (Glas) and Pathologists (UK). In the outbreak of avian influenza virus H5N1 in 1997 in Hong Kong, Professor Yuen was the first to report in Lancet about the unusual clinical severity and high mortality of infected patients which could be identified by the in-house designed molecular test at his laboratory. During the outbreak of SARS in 2003, he led his team in the discovery of the SARS coronavirus. Subsequently he found the natural reservoir of SARS coronavirus like virus in Chinese horseshoe bat. His success in finding novel microbes is exemplified by the discovery of more than 40 viruses, 10 bacteria, 2 fungi and 2 protozoa in human and animals. His 600 publications with over 15,000 citations are mainly related to the research of novel microbes or emerging infectious disease agents.

# PROGRAMME

## 11 December 2014 (Thursday – Day 1)

| | |
|---|---|
| 0800 – 0830 | Registration |
| 0830 – 0900 | Opening (*Note 1*) |
| 0900 – 0915 | Photo-taking Session |

### SESSION 1 – REPRODUCTIVE GENETICS

0915 – 1000 *Keynote Lecture*

*Chairperson:* *Daniel H.S. LEE (Hong Kong)*

**Dennis Y.M. LO** *(Hong Kong)*

Non-invasive prenatal testing using fetal DNA in maternal plasma: a diagnostic revolution in progress [P. 9]

*Chairpersons:* *Po Sing LEUNG (Hong Kong) &*
*Chanvit LEELAYUWAT (Khon Kaen)*

1000 – 1025 **Richard K.W. CHOY** *(Hong Kong)*
Utilization of whole genome sequencing technique in human embryos prior to implantation [P. 13]

1025 – 1050 **Hsiao Chang CHAN** *(Hong Kong)*
Deficient HBD-1 underlies male infertility associated with poor sperm motility and genital tract infection [P. 14]

1050 – 1115 **Virasakdi CHONGSUVIVATWONG** *(Hat Yai)*
Development and use of open-source software and packages in health research and research training: twelve-year experience of Prince of Songkla University [P. 15]

1115 – 1135 Tea Break & Poster Session

### SESSION 2 – ONCOLOGY

*Chairpersons:* *Franky L. CHAN (Hong Kong) &*
*Dang Duc LONG (Danang City)*

1135 – 1200 **Andrew M.L. CHAN** *(Hong Kong)*
Cancer cell signaling: Mechanistic insights into a tumor suppressor [P. 16]

1200 – 1225 **Dang Duc LONG** *(Danang City)*
Combining multiple biomarkers in cancer diagnosis [P. 17]

1225 – 1250 **Chi Fai NG** *(Hong Kong)*
Current roles of biological markers in the management of prostate cancer [P. 18]

1250 – 1315 **Hean OOI** *(Taichung)*
The metabolic characteristic changes of F-18 fluorodeoxyglucose uptake in advanced lung adenocarcinoma with common and uncommon epidermal growth factor receptor mutations [P. 19]

1320 – 1430 Welcome Lunch

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| | **SESSION 3 – STEM CELL** |
| 1435 – 1520 | *Keynote Lecture*<br>*Chairperson: Wing Ho YUNG (Hong Kong)*<br>**Yoo Hun SUH** *(Daegu)*<br>Pathogenesis and therapy for Alzheimer's disease [P. 10] |
| | *Chairpersons: Kenneth K.H. LEE (Hong Kong) & Chatchai TAYAPIWATANA (Chiang Mai)* |
| 1520 – 1545 | **Chatchai TAYAPIWATANA** *(Chiang Mai)*<br>Combating HIV by novel stem cell gene therapy using ankyrin and zinc finger protein [P. 20] |
| 1545 – 1610 | **Bo FENG** *(Hong Kong)*<br>Direct activation of pluripotency genes using engineered TALE and Cas9 transcription factors [P. 21] |
| 1610 – 1630 | Tea Break & Poster Session |
| 1630 – 1655 | **Patcharee JEARANAIKOON** *(Khon Kaen)*<br>Discrimination of micromass-induced chondrocytes from human mesenchymal stem cells by focal plane array-fourier transform infrared microspectroscopy [P. 22] |
| 1655 – 1720 | **Kingston K.L. MAK** *(Hong Kong)*<br>The molecular control of fracture healing and bone regeneration [P. 23] |
| 1720 – 1830 | **COMPETITION FOR THE "ASIA-INTERNATIONAL BIOMEDICAL SCIENCE CONSORTIUM – OUTSTANDING POSTER AWARD"**<br>(Poster Presentation and Q&A Session) |
| 1830 – 1930 | Welcome Reception |

*Note 1: Including welcoming speech by (1)* ***Fanny M.C. CHEUNG****, Pro-Vice-Chancellor, The Chinese University of Hong Kong; (2)* ***Andrew YOUNG****, Vice President, Marketing & Sales of Hong Kong Science and Technology Parks Corporation; (3)* ***Wai Yee CHAN****, Director, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong*

## 12 December 2014 (Friday – Day 2)

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| | **SESSION 4 – INFECTIOUS DISEASES & IMMUNOLOGY** |
| 0830 – 0915 | *Keynote Lecture*<br>*Chairperson: Paul K.S. CHAN (Hong Kong)*<br>**Kwok Yung YUEN** *(Hong Kong)*<br>The lingering MERS versus the explosive SARS [P.11] |
| | *Chairpersons: Stephen K.W. TSUI (Hong Kong) & Boon Huat LIM (Pulau Pinang)* |
| 0915 – 0940 | **Boon Huat LIM** *(Pulau Pinang)*<br>Diagnosis of neglected tropical diseases [P. 24] |
| 0940 – 1005 | **Noraziah Mohamad ZIN** *(Selangor)*<br>Molecular diagnosis of tuberculosis: The Malaysian experience [P. 25] |
| 1005 – 1030 | **Paul K.S. CHAN** *(Hong Kong)*<br>Human papillomavirus: A ubiquitous virus with emerging health concern [P. 26] |
| 1030 – 1050 | Tea Break & Poster Session |
| 1050 – 1135 | *Keynote Lecture*<br>*Chairperson: Yu HUANG (Hong Kong)*<br>**Tse Wen CHANG** *(Taipei)*<br>Rational drug design: Antibodies targeting the IgE pathway for treating severe asthma and chronic urticarial [P. 12] |
| | *Chairpersons: Tzi Bun NG (Hong Kong) & Pei Pei CHONG (Selangor)* |
| 1135 – 1200 | **Yupin SUPUTTAMONGKOL** *(Salaya)*<br>Disseminated non-tuberculous mycobacterial infection and acquired anti-interferon-γ autoantibody in previously healthy adult patients [P.27] |
| 1200 – 1225 | **Pei Pei CHONG** *(Selangor)*<br>Insights into host responses against infections by Candida species from transcriptional profiling [P. 28] |
| 1225 – 1250 | **David S.C. HUI** *(Hong Kong)*<br>The role of immune-modulating agents for severe influenza [P. 29] |
| 1250 – 1305 | **"ASIA-International Biomedical Science Consortium – Outstanding Poster Award" Presentation Ceremony** |
| 1310 – 1425 | Lunch |
| | **COMPETITION FOR THE BEST STUDENT PRESENTATION AWARDS IN THE SCHOOL OF BIOMEDICAL SCIENCES, THE CHINESE UNIVERSITY OF HONG KONG** |
| 1430 – 1530 | Student Oral Presentations 1 – 4 |
| 1530 – 1600 | Tea Break & Poster Session |
| 1600 – 1730 | Student Oral Presentation 5 – 10 |
| 1730 – 1800 | School of Biomedical Sciences Postgraduate Research Day Best Posters Presentation Ceremony |
| 1800 – 2100 | Conference Banquet |

# Keynote-01

## Non-invasive prenatal testing using fetal DNA in maternal plasma: a diagnostic revolution in progress

Y.M.D. Lo

Li Ka Shing Institute of Health Sciences and Department of Chemical Pathology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, P.R. China.

The discovery of cell-free fetal DNA in maternal plasma in 1997 has opened up a spectrum of possibilities for non-invasive prenatal testing. The last few years have seen this technology being rapidly translated for fetal chromosomal aneuploidy detection in a global manner. There are a number of exciting new developments that would shape the progress of this field in the coming few years. First, recent data have shown that apart from fetal genome sequencing, one can also determine the fetal methylome from maternal plasma. Second, genomewide transcriptome profiling using massively parallel RNA sequencing in maternal plasma has also been recently achieved. Third, developments of targeted sequencing approaches have indicated the robustness of this method for the non-invasive prenatal diagnosis of a number of monogenic diseases. Hence, it is likely that non-invasive prenatal testing will be increasingly applied in the clinical delivery of prenatal care.

# Keynote-02

## Pathogenesis and therapy for Alzheimer's disease

Y.H. Suh

Korea Brain Research Institute, South Korea.

The APP-CTs (CT 99, AICD [C57, C59] and C31) have been found in the brains of patients with Alzheimer's disease (AD). Here, we demonstrate for the first time that the APP-CTs exert neurotoxicity on differentiated PC 12 cells and rat primary cortical neurons by inducing the expression of glycogen synthase kinase 3beta, forming a ternary complex with Fe65 and CP2/LSF/LBP1 in the nucleus, whereas deletion mutants and a point mutant with Y682G of the YENPTY domain, a Fe65 binding domain, do not. Moreover, expression of APP770 and Swedish mutant form of APP increased the levels of C-terminal fragments of APP (APP-CTs) in neuronal cells and also induced the up-regulation of glycogen synthase kinase-3beta at both the mRNA and the protein levels. In addition, we show that CP2/LSF/LBP1 binding site (nt +0 to approximately +10) in human glycogen synthase kinase 3beta promoter region is essential for the induction of the gene transcription by APP-CTs.

In addition, here, we report that phosphorylation at APP-CT▪Thr668 regulates the nuclear translocalization of APP-CTs by affecting the interaction of APP-CT and Fe65. And APLP2-CTs can affect GSK-3β transcription by forming the ternary complex, suggesting that APLP2 plays a role in tau phosphorylation in the pathogenesis of AD.

Neuroinflammation is one of the important features in the pathogenesis of Alzheimer’s disease (AD), which is the most prevalent neurodegenerative disorder.

To investigate the regulatory genes responsible for the neuropathology in AD, we performed microarray analysis with APPV717I-CT100 transgenic mice and isolated the S100a9 gene. We also found that S100a9 expression was increased in the brains of Tg2576 mice, animal model of AD and AD patients. Silencing of S100a9 gene decreased the production of inflammatory cytokines induced by Aβ or APP-CTs in BV2 cells. In Tg2576 mice, knockdown of S100a9 gene significantly reduced the neuropathology and improved the learning ability. These results clearly show that the upregulation of S100a9 gene plays an important role in the neuropathology and memory impairment in AD, suggesting that the regulation of this gene has a therapeutic potential for AD.

Recent studies of stem cell show its therapeutic potential for neurodegenerative disorders by differentiating into other cell lineages and replacing damaged cells. Among stem cells, peripheral stem cells are readily accessible and autologous stem cell transplantation would have no immune rejection responses.

Here, we used human adipose-derived stem cells (hASCs) and examined whether intracerebrally or peripherally transplanted human ASCs could have therapeutic or preventive effects in AD mouse model (Tg2576). hASCs were stereotaxically transplanted to hippocampus regions of Tg2576 mice. Or tail vein injection of hASCs was started at 3 months of age. We performed the Morris water maze task at 14 months of age and we found that the memory impairment in Tg2576 mice was actually improved by injection of hASC. One month later, animals were sacrificed to observe pathological changes. Congo red staining showed decreased number of plaques in cortex and hippocampus of hASC-injected Tg mice brains compared to those of Tg-sham mice.

We demonstrate with these data that intracerebral or pheripheral injection of hASCs rescue memory deficit and give benefits to blocking the pathogenesis in the brain of Tg mice by reducing the number of plaques. Hence, we demonstrate that hASCs are expected to be preventive and therapeutic approach for AD though further studies should be done.

## Keynote-03

### The lingering MERS versus the explosive SARS

K.Y. Yuen

Department of Microbiology and State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong SAR, P.R. China.

The explosive epidemic of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) within 6 months in 2003 has affected over 8000 patients with 10% fatalities. The source of this epidemic was traced to wild life market civets and raccoon dogs, and finally to the Chinese horseshoe bats (Rhinolophus sinicus). This important finding has sparked an intensive hunt for novel coronaviruses in human and animals. Novel human coronaviruses NL63 and HKU1 were soon discovered in patients with acute febrile respiratory illness with no known causes. At least another 40 animal coronaviruses were found in bats, birds and other domestic or wild animals including dolphins, of which 23 were found in Hong Kong. Notably in 2006, the bat coronavirus HKU4 was discovered in the lesser bamboo bat (Tylonycteris pachypus) and bat coronavirus HKU5 was found in Japanese pipistrelle bat (Pipistrelle abrams) with their virus genomes fully sequenced. Since 2012, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) has affected over 800 patients with 34% mortality after emerging in Saudi Arabia. This novel virus is phylogenetically closely related to the bat coronavirus HKU4 and HKU5. All these MERS patients have either resided in or travelled to the Middle East. Only few cases had clear exposure history to camels while secondary cases were often traced to health care facilities. The natural reservoir of MERS-CoV remains elusive but many camels have been found to be shedding of MERS-CoV in the nasopharynx or have high serum antibody titre against the virus which could serve as the continuous source of animal-to-human transmission. Unlike SARS, more patients with severe MERS were elderly, male and had underlying medical co-morbidities especially diabetes and chronic renal failure. Moreover the high fatality of MERS-CoV may be related to its broad tissue tropism and ability to infect macrophages and dendritic cells leading to impairment of innate immune response and cytokine dysregulation. Young healthy contacts of MERS patients can be asymptomatic or mildly symptomatic. No specific antiviral agent is yet tested in randomized control trial and therefore treatment is mainly supportive. Extracorporeal membrane oxygenation and renal replacement therapy are often necessary in severe cases. Robotic screening for commercially available antivirals showed that betaferon and mycophenolate are effective in cell culture with much better pharmacokinetics than the combination of interferon alpha and ribavirin which appeared effective in a monkey model of MERS. Convalescent plasma with neutralizing antibody was used for anecdotal treatment of SARS but in vitro study suggested the possibility of immune enhancement if adequate antibody titer cannot be achieved. Potent neutralizing human monoclonal antibody against MERS-CoV can be selected from single-chain variable region fragments of a non-immune human antibody library. Very potent virus-host cell membrane fusion inhibitor against MERS-CoV entry into cell can also be made from antiviral peptides directed against the surface Spike protein of MERS-CoV.

## Keynote-04

### Rational drug design: Antibodies targeting the IgE pathway for treating severe asthma and chronic urticaria

T.W. Chang

Genomics Research Center, Academia Sinica, Taiwan.

In this lecture, the speaker will share his experience in creating a new therapeutic concept and bringing it through laboratory research, drug optimization processes, clinical trials, and finally to broad patients' use. In the beginning of this lengthy drug development program, free IgE in blood and membrane-bound IgE (mIgE) on B lymphocytes were recognized as therapeutic targets, when other researchers did not consider that these molecules were suitable targets or that they were targetable. Today, the anti-IgE antibody, omalizumab (trade name Xolair) has been studied in more than 120 clinical trials for various allergic diseases, including asthma, allergic rhinitis, food allergy, etc. and some non-allergic diseases, especially, skin diseases. Xolair has been approved in more than 90 countries to treat patients with severe allergic asthma untreatable with other medicine. Xolair has also been approved in 2014 in the European Union and the U.S.A. for treating recalcitrant chronic spontaneous urticaria untreatable with H1-antihistamines. Chronic spontaneous urticaria is mainly an autoimmune disease, which involves mast cells. New-generation drug candidates for targeting mIgE, which may have certain advantages over omalizumab, have been developed and one of those is in Phase IIb clinical trial. Those antibody drugs targeting the IgE pathway have not only provided relief for patients with severe allergic diseases and mast cell-mediated non-allergic diseases but also helped understanding the pathological mechanisms of those diseases. The editor of Nature Biotechnology commented that these programs were good examples of rational drug design approach.

# Oral-01

## Utilization of whole genome sequencing technique in human embryos prior to implantation

R.K.W. Choy

Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China.

Chromosomal aberrations including numerical, structural and balanced chromosome rearrangement (BCA) is a common cause of infertility and recurrent miscarriage. Microarray based comprehensive chromosome screening methods, applicable to single cells biopsied from preimplantation embryos, allow reliable identification and transfer of euploid embryos. Recently, randomized trials using such methods have indicated that aneuploidy screening improves IVF success rates. However, the high cost of testing and inability of detecting structural and BCA has restricted the availability of this potentially beneficial strategy. We have demonstrated that next generation sequencing (NGS) at a low-coverage sequencing approach to detect numerical and balance chromosomal abnormality events independent of knowing the affected regions enable a comprehensive chromosome analysis. In this study we attempt to address can NGS techniques be used reliably for comprehensive aneuploidy screening of human embryos from patients undergoing IVF treatments, with the purpose of identifying and selecting chromosomally normal embryos for transfer.

## Oral-02

### Deficient HBD-1 underlies male infertility associated with poor sperm motility and genital tract infection

R.Y. Diao[1,2], K.L. Fok[1], H. Chen[1,2], Z.M. Cai[2], H.C. Chan[1]

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Human β-defensin 1 (DEFB1, also named HBD-1) is the first identified member of the β-defensin family with antimicrobial activities and wide distribution in various epithelia throughout the body, including the epididymis. Recent studies have shown that DEFB1 is not only expressed in the epithelium of the epididymis, but also present in seminal plasma and ejaculated spermatozoa, indicating that DEFB1 secreted by the epididymis is able to bind to sperm. However, its role in regulating sperm function and defending male fertility has not been explored. Genital tract infection and reduced sperm motility are considered two pivotal etiological factors for male infertility associated with leukocytospermia and asthenozoospermia, respectively. Our recent studies have demonstrated that the expression level of DEFB1 in sperm from infertile men exhibiting either leukocytospermia or asthenozoospermia, both of which are associated with reduced motility and reduced bactericidal activity in sperm, is much lower compared to that in normal fertile sperm. Interference with DEFB1 function also decreases both motility and bactericidal activity in normal sperm, whereas treatment with recombinant DEFB1 markedly restores DEFB1 expression, bactericidal activity, sperm quality, and egg-penetrating ability in sperm from both asthenozoospermia and leukocytospermia patients. DEFB1 triggers $Ca^{2+}$ mobilization, which is important for sperm motility, through its interaction with chemokine receptor type 6 (CCR6) in sperm. Interference with CCR6 function also reduces motility and bactericidal activity of normal sperm. These results have revealed a common defect in male infertility associated with both asthenozoospermia and leukocytospermia, indicating a dual role of DEFB1 in defending male fertility and providing a feasible therapeutic approach for male infertility associated with poor sperm motility and genital tract infection.

## Oral-03

### Development and use of open-source software and packages in health research and research training: Twelve years experience of Prince of Songkla University

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Research capacity and development of human resources for health research are utmost important issues in developing countries. Epidemiology Unit of Prince of Songkla University started an international programme for graduate study since 1992 with a mission on “Human Resources of Health Research”. In its first decade, student teaching and research were based on purchasing proprietary statistical software. When the alumni returned to their home institutes, the use of this software became illegal and the copies easily spread in the countries where the students came from. In 2002, we changed to use “R”, which is open-source. It is however difficult to learn and to use. We then developed a package ‘epicalc’ based on R which makes R easier to explore data, more powerful in data management and produce outputs that are manuscript ready. In 2006, this package has been made for global free access and use at the website for Comprehensive R Archive Network <cran.r-project.org>, where we also published a free tutorial book for self-study <cran.r-project.org/doc/contrib/Epicalc_Book.pdf>. For more than a decade, we have published hundreds of research manuscripts in indexed journals using this software and package without any problem. They are also used and taught at institutes in other countries. The part of the package on sample size calculation was used to create a free app for on App Store of iOS and Play Store of Android phone and tablets under the name “n4Studies” to make it even morehandy and easier to use by researcher with relatively little statistic background. The overall strategy of open-source base thus has not only solved the problems of illegal software but also increase research capacity in both developed and developing countries.

## Oral-04

### Cancer cell signaling: Mechanistic insights into a tumor suppressor

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Human carcinogenesis is initiated by a host of driver mutations and the stochastic accumulations of additional mutations to attain full malignancy. Among the numerous cancer-related genes discovered so far, the loss of tumor suppressor genes is one of the key rate-limiting steps during tumor progression. *PTEN* is a major tumor suppressor gene inactivated in over 30% of human cancer. Germ line mutations in the *PTEN* gene is the genetic cause of the Cowden Disease syndrome which predisposes carriers to breast cancer. The *PTEN* gene encodes a lipid phosphatase and its subcellular localization and enzymatic activity are regulated by its carboxyl-terminal tail region. This tail region is involved in epithelial junction integrity and frequently deleted in Cowden Disease as well as in malignant brain tumors. A mouse strain was generated to probe for the functions of this domain in tumorigenesis. This mouse line develops normally without gross phenotypic aberrations. However, haploinsufficent mice have significantly greater brain mass with other organs not being affected. In addition, when mated to an MMTV-PyMT breast cancer model, homozygous knockout mice promote breast carcinogenesis with greater tumor mass and metastasis. The significance of these results will be discussed.

# Oral-05

## Combining multiple biomarkers in cancer diagnosis

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Incidence and mortality from cancer in Vietnam are increasing at an alarming rate and now among the highest rates in the world. Accurate and early detection of cancer is an important action to stem this increase. Here we explore the combination of serum biomarkers for differentiating breast cancer from benign. Fifty two females (27-70 years), diagnosed as early breast cancer patients with or without metastasis, and sixty healthy individuals were selected for this study. The biomarkers under investigation were miR-155, miR-195, miR-21, and C-reactive protein (CRP). Four classification methods (t-test, logistic regression, linear discriminant analysis, random forest) that combines these biomarkers have been developed and evaluted. The 4 marker combination from the logistic regression showed the best performance against the other selection methods in terms of the sensitivity and specificity. This combination is also shown to have significant improved performance compared to measuring of specific antigens and/or biopsy.

## Oral-06

### Current roles of biological markers in the management of prostate cancer

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Prostate cancer (PCa) has becoming an increasing important health care problem in Asian population in the past decades. Currently, it is ranked number three in the incidence of male cancer in Hong Kong. The use of serum prostate specific antigen (PSA) has greatly improved the diagnosis of early PCa and about 2/3 of our prostate cancer patients was now diagnosed by PSA-based care. However, the low specificity of PSA for PCa diagnosis inevitably resulted in many unnecessary stress and morbidities to patients during the workup of patients with elevated serum PSA level. Because of the diversity in the natural history of early PCa, some patients with low risk cancer may not necessary need to have invasive treatment, i.e. adopting the active surveillance approach. Currently there is a long list of biomarkers developed to attempt to improve the diagnosis and also stratification risk profile PCa patients. In this review, common new markers for diagnosis, risk stratification and prognostic prediction would be discussed and also local experience in the usage of these markers will be shared.

## Oral-07

### The metabolic characteristic changes of F-18 fluorodeoxyglucose uptake in advanced lung adenocarcinoma with common and uncommon epidermal growth factor receptor mutations

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**Objective:**
Epidermal growth factor receptor (EGFR) mutations in lung adenocarcinoma are involved in the regulation of tumor metabolism. F-18 fluorodeoxyglucose-positron emission tomography ([18F]FDG PET), is a functional molecular imaging, it used in clinically to measure metabolic of [18F]FDG PET uptake in cancer. Thus, we hypothesize that there will exist difference metabolic uptake in between common and uncommon EGFR mutation in the lung adenocarcinoma.

**Methods:**
From May 2010 to April 2013, patients with stage IIIB or IV lung adenocarcinoma who underwent [18F]FDG PET and EGFR mutation analysis before receiving any treatment were eligible to participate in this study. Standard uptake value(SUV), metabolic tumor volume(MTV) and total lesion glycolysis (TLG) of each malignant lesion were measured. Whole body MTV and whole body TLG were the summation of all the MTV and TLG values in every cancer. The association of wild type, common and uncommon EGFR mutation status with patient characteristics were evaluated.

**Results:**
Of the 180 advanced adenocarcinoma, 12 were uncommon EGFR mutation, 117(65%) were common EGFR mutation, and 51 were wild type. The wild type showed significantly higher whole body MTV and TLG but not difference in organ involved and lymph node metastasis. In a subgroup analysis of EGFR mutation, uncommon mutation (other than deletion 19 or L858R) shown significantly higher whole body TLG but not MTV when compared to common mutation (deletion 19/L858R). In the another subgroup analysis of uncommon EGFR mutation, the combination uncommon EGFR mutation without deletion 19 or L858R showed significantly difference in TLG and MTV uptake, more organ involved (> 3 organ) and lymph node metastasis compared to those uncommon EGFR mutation mixed with deletion 19 or L858R.

**Conclusions:**
The wild type had a higher glucose metabolism when compared to those genotypes in overall patients. The different metabolic activity was noted in common and uncommon EGFR mutation. Uncommon EGFR mutation represents higher glycolysis activity and more advanced metastasis, which suggests tumor was aggressiveness without any deletion 19 or L858R mutation.

## Oral-08

### Combating HIV by novel stem cell gene therapy using ankyrin and zinc finger protein

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Anti-HIV gene delivery has been proposed as a salvage approach for multi-drug resistant individual and as a less toxic, long-term replacement for HAART. Recently, novel designed molecular scaffolds have been established to interfere HIV life cycle. 2LTRZFP, designed zinc finger protein, was designed to target 2-LTR-circle junction of HIV-1 DNA for blocking viral integration into host chromosome. In addition, AnkGAG1D4, ankyrin-repeat protein, was developed to target HIV-1 capsid for disrupting viral assembly. Remarkably, these novel gene therapy approaches showed drastic reduction of HIV-1 replication in T cell lines. Our strategy in this study is based on applying of stability of anti-HIV-1 effects of these novel designed scaffolds in induced pluripotent stem cells (iPSCs) by using lentiviral vector system. These iPSCs can be maintained the pluripotentcy as embryonic stem cells and have enormous potential as a source of autologous cells for therapeutic use to eliminate the problem of graft rejection. Anti-HIV HSC derived iPSCs have ability to renew and generate many hematopoietic cell types including T lymphocytes, macrophages, and dendritic cells to resist HIV-1 infection. Moreover, the anti-HIV iPSC lines can be frozen and stored for future use. The modified CD34+ mobilized human peripheral blood cells or fibroblast will be used as the starter cells for generating iPSCs by transducing Oct4, Sox2, Klf4, and c-MYC reprograming factors into the stem cells containing anti-HIV genes. Then, the iPSC clone that contains 2LTRZFP or AnkGAG1D4 will be differentiated toward hematopoietic progenitor cells (HPC) and finally T lymphocytes or macrophages in vitro. Then anti-HIV HSC derived iPSC will be transplanted to humanized mice, NSG strain, to study T lymphocyte and macrophage developments in vivo. To test the ability of the modified T lymphocytes or macrophages to inhibit HIV-1 replication, iPSC-derived T lymphocyte and macrophage will be challenged with HIV-1 both in and ex vivo to test the anti-HIV activity by measuring a reduction of p24 level and viral load comparing with uncontained anti-HIV gene control group. Thus, our novel designed scaffolds in iPSCs could potentially serve as an alternative treatment for HIV-infected individuals and would help overcome the disadvantages of current HIV therapy procedures. The data generate from this proposal will bring the novel therapies for curing HIV closer to the clinic and applying for translational medicine.

## Oral-09

### Direct activation of pluripotency genes using engineered TALE and Cas9 transcription factors

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The newly developed TALE and CRISPR/Cas9 transcription factors (TF) offered a powerful and precise approach for modulating gene expression. In this study, we systematically investigated the potential of these new tools in activating the stringently silenced pluripotency gene *Oct4 (Pou5f1)* in mouse and human somatic cells. First, with a number of TALEs and sgRNAs targeting various regions in the mouse and human *Oct4* promoters, we found that the most efficient TALE-VP64s bound around −120 to −80 bp, while highly effective sgRNAs targeted −147 to −89 bp upstream of the transcription start sites (TSS) to induce high activity of luciferase reporters. In addition, we observed significant transcriptional synergy when multiple TFs were applied simultaneously. Although individual TFs exhibited marginal activity to up-regulate endogenous gene expression, optimized combinations of TALE-VP64s could enhance endogenous *Oct4* transcription up to 30-fold in mouse NIH3T3 cells and 20-fold in human HEK293T cells. More importantly, the enhancement of *OCT4* transcription ultimately generated OCT4 proteins. Furthermore, examination of different epigenetic modifiers showed that histone acetyltransferase p300 could enhance both TALE-VP64 and sgRNA/dCas9-VP64 induced transcription of endogenous *OCT4*. Taken together, our study suggested that engineered TALE-TF and dCas9-TF are useful tools for modulating gene expression in mammalian cells.

## Oral-10

### Discrimination of micromass-induced chondrocytes from human mesenchymal stem cells by focal plane array-fourier transform infrared microspectroscopy

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To date, most of the standard protocols for determining stem cell differentiation are laborious and involve cell isolation, fixation and subsequent specific staining. Specialized expensive reagents, as well as stem cell samples, are consumed during multiple testing procedures. Rapid and sensitive methods for evaluation of stem cell differentiation are required for facilitating future stem cell therapies.

We aimed to evaluate the capability of Synchrotron Radiation Fourier Transform Infrared (SR-FTIR) and Focal Plane Array-Fourier Transform Infrared (FPA-FTIR) microspectroscopy for characterising the differentiation of chondrocytes from human mesenchymal stem cells (hMSCs). Collagen and aggrecan expression were analyzed to monitor the successful induction in parallel with the spectroscopy by reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis.

Spectral signature derived from chondrocyte-induced cells revealed strong IR absorbance bands attributed to collagen near 1338 and 1234 $cm^{-1}$ and proteoglycan at 1245 and 1175-960 $cm^{-1}$ compared to the non-induced cells. In addition, spectra from control and induced cells are clearly segregated into separate clusters using Partial Least Squares Discriminant Analysis (PLSDA) scores plots at the early induction stages with 100% accuracy by both SR-FTIR and FPA-FTIR. The spectral signature for this discrimination was attributed with collagen and aggrecan protein in concordant with those obtained from RT-PCR and western blot data.

In conclusion, both SR-FTIR and FPA-FTIR microspectroscopy have the capability for stem cell characterization allowing rapid and sensitive detection of biomolecular changes during chondrogenic differentiation. FTIR microspectroscopy exerts as a label-free tool which can facilitate stem cell therapies in a low resource setting.

## Oral-11

### The molecular control of fracture healing and bone regeneration

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Bone possesses intrinsic repair capacity for regeneration in response to fracture injury. Although many signaling activities in endochondral and intramembranous ossifications are recapitulated during the repairing process, osteogenesis is less efficient suggesting that additional signaling cues are employed for regeneration. Hippo signaling controls organ size and tissue regeneration in many organs, but its roles in bone repair remain elusive. Here, we demonstrate that Yap1, an effector of Hippo pathway, governs the initiation of fracture repair by regulating cartilage maturation. Yap1 activation in mice shows severely impaired cartilaginous callus formation after fracture injury, but skeletal development of these mice are relatively normal. Mechanistically, Yap1 regulates chondrocyte differentiation at multiple steps during bone repair. First, Yap1 is required for mesenchymal stem cell maintenance. It also promotes early chondrocyte proliferation but inhibits further chondrocyte maturation. Our results identify Hippo pathway as a specific regulator responsible for the initiation of endogenous bone repair and it could be a potential therapeutic target for treatment of fracture injury.

# Oral-12

## Diagnosis of neglected tropical diseases

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Neglected tropical diseases (NTDs) are a group of 17 or more infectious diseases, which thrive in resource-limited settings and afflict the bottom billions of the world population. NTDs are reported in 149 countries, in which two or more diseases are endemic in over 100 countries. Among the established 17 NTDs, eight are caused by helminths; three by protozoa; four due to bacteria and two by viruses. NTDs cause morbidity and death to poor and disadvantaged populations who have low visibility and little political voice. Co-infection with NTD can exacerbate the two other widespread killer diseases such as tuberculosis and HIV/AIDS, which are also synonymous with poverty. Generally, NTDs do not spread far and wide but cause stigma and discrimination especially to females. The diseases are expected to be controlled, prevented and possibly eliminated using effective and feasible solutions; however researches on the diseases are still relatively neglected.

Conventional laboratory diagnostic tests are still being widely used to screen NTDs in endemic populations; and the inherent lack of test sensitivity often lead to undetected patients becoming active sources of infections. Preventive chemotherapy is deemed a more effective approach to control NTDs. Nonetheless, the effectiveness of chemotherapies in endemic population has to be monitored by utilizing sensitive and specific diagnostic tests. Nucleic acid amplification assays may fulfill the two pertinent diagnostic criteria but such quality assays are either costly or heavily dependent on equipment, which discourage their applications in low-resource settings where basic amenities such as electricity are often unavailable. Unfortunately, the number of commercially available diagnostics for NTDs that fulfilled the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free and Deliverable) criteria are either non-existence or too few.

In conclusion, development of ASSURED diagnostics requires the concerted and committed efforts of biomedical scientists from both developing and developed countries. Research scientists and industries have to pull their expertise and resources from disciplines such as pathogen molecular biology, pathogen immunology, microfluidics, bioinformatics and electronic engineering to make available quality diagnostics to reduce the unceasing threats of NTDs to the bottom billions of the world population.

# Oral-13

## Molecular diagnosis of tuberculosis: The Malaysian experience

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Detection of *Mycobacterium tuberculosis* may be difficult while available conventional methods have their limitations. Early detection of TB caused by this bacterium might contribute in lowering the transmission risk of infection. A study was designed to demonstrate the molecular diagnosis usefulness for direct detection of *M. tuberculosis* from clinical samples. A method of Duplex Polymerase Chain Reaction (DPCR) with primer specific for detection of the IS*6110* insertion element of *M. tuberculosis* complex and the p*53* gene of human beta globin (Hbb) were developed. Then, electrochemical biosensor was used as an alternative method for detection of the product of Polymerase Chain Reaction (PCR). The focus was to utilize pencil graphite electrode (PGE) as the working electrode whereas ruthenium bipyridine as hybridization indicator. *M. tuberculosis* synthetic oligonucleotides were used to study the DNA hybridization event on PGE. Various parameters affecting the response of differential pulse voltammetry (DPV) signal were explored and optimized including concentration of probe, immobilization time probe, concentration of target, hybridization time, concentration of hybridization indicator and accumulation time of indicator. Clinical samples from patients suspected with *M. tuberculosis* infection were used. DPCR was able to identify 44% of positive samples in which acid fast bacilli (AFB) and culture were negative. In addition, 95% of the AFB positive specimens can be detected by DPCR. As for the biosensor, optimization study showed that the optimum concentration and time immobilization of probe was at 10 µg/ml and 20 minutes. The optimum concentration of target to bind completely to the probe was 15 µg/ml, hybridization time was 9 minutes whereas the indicator to accumulate was 5 minutes. The process was continued using synthetic oligonucleotide and clinical sample obtained from DPCR product. The voltammetric signal that was produced by positive and negative *M. tuberculosis* samples were 2.278 – 2.366 µA and 3.857 – 3.903 µA, respectively. The results obtained showed that the hybridization event between probe and positive *M. tuberculosis* samples yields higher voltammetric signal whereas the hybridization between probe and negative *M. tuberculosis* samples yields lower voltammetric signal. In conclusion, this study showed that the DPCR and biosensor can be applied as an alternative method to detect the presence of *M. tuberculosis* DNA in clinical samples.

## Oral-14

### Human papillomavirus: A ubiquitous virus with emerging health concern

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To date, about 200 different types of human papillomavirus (HPV) have been identified. HPV infects keratinocytes and cause lesions over the mucosal and cutaneous surface of the body. The virus is mainly transmitted by direct contact with infected skin or mucosal surface. The anogenital group of HPV is mainly transmitted by sexual contact. Inanimate objects probably play a role as the virus is expected to be quite resistant. The consequent of infection ranges from completely asymptomatic to cancer development. Almost everyone has had HPV infection. A survey conducted in Hong Kong showed that 1 in 12 adult women are carrying HPV in their cervix. HPV is well-known as a necessary but insufficient factor for the development of cervical cancer. The knowledge on aetiological role of HPV in cervical cancer has been translated successfully into clinical use. HPV test has been adopted as one of the first line screening test or used as an adjunct test upon the detection of abnormities based on cytological examination. Two highly effective prophylactic vaccines covering the two major cancer-associated types are available. With the improved tools for screening and the newly available prophylactic vaccines, the incidence of cervical cancer is expected to further decrease in countries that can afford these preventative strategies. However, another previously unrecognized HPV-associated cancer has emerged over the last century. The overall incidence of head and neck cancers have been on the decreasing trend in most developed countries where a certain success in controlling smoking have been achieved. Paradoxically, one subset of head and neck cancers is on the raising trend. These are oropharyngeal cancers which have been shown to associate with HPV. The pendulum is now shifting. For instance, in the United States, the recent incidence of HPV-associated cancers in man is higher than that of women. Several lines of evidence also suggest a role of HPV in skin cancer which is one of the most common cancers in Europe.

## Oral-15

### Disseminated non-tuberculous mycobacterial infection and acquired anti-interferon-γ autoantibody in previously healthy adult patients

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A newly identified clinical syndrome of disseminated non-tuberculous mycobacterial (dNTM) infection in patients who previously healthy is now well recognized in association with an acquired autoantibody to interferon-gamma (IFN-γ). This syndrome is emerging as an important cause of morbidity and mortality among Asian population.

We identified 54 previously healthy adults who developed this syndrome and presented to Siriraj Hospital, Bangkok, Thailand between October 2006 and August 2014. The antibody to IFN-γ in the serum was determined by enzyme-linked immunosorbent assay (ELISA). The optical density (O.D.) of greater 1 was defined as positive for antibody to IFN-γ in this population. Two patients died before enrollment and the anti- IFN-γ status was unknown. Among 52 patients who had at least one anti IFN-γ level tested, 27 (52%) were female with a mean ±SD age of 50±12 years. Twenty seven patients had active dNTM disease when their anti-IFN-γ concentrations were first measured. The initial mean ±SD concentrations of anti-IFN-γ in group of patient with active and in group of patient with stable or non-active disease were 3.88±0.73 O.D. and 3.08 ±1.03 O.D. respectively (p=0.02). Forty-four patients were followed up for a mean duration of 1 year (range between 1 to 28 months). Thirty-six (69.2%) patients were clinically stable either with or without maintenance antimycobacterial therapy, 6 (11.5%) patients had active diseases and one patient died from uncontrolled NTM disease. Anti-IFN-γ remained detectable or persisted in all patients during follow up. Our preliminary analysis showed that both initial and latest concentration of anti-IFN-γ were significantly higher in group of patients who developed recurrent or remained in active disease at follow up (4±0.5 O.D and 4.19±0.21O.D.) than in group of patients who had stable diseases after treatment (3.43±1 O.D. and 3.71±0.76 O.D., p=0.03 and p=0.01 respectively). At present this study is ongoing in order to determine kinetic of anti-IFN-γ concentrations and other risk factors associated with outcome of antimycobacterial treatment or resolution of infection.

## Oral-16

### Insights into host responses against infections by Candida species from transcriptional profiling

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Fungi of the Candida species are medically important, opportunistic pathogens that cause a wide range of infections in immunocompromised hosts such as transplant patients, HIV-positive individuals, and cancer patients. The spectrum of infections caused by these fungi includes not only superficial or mucosal infections, but also life-threatening disseminated, deep-seated organ infections and invasive infections. Throughout the past few decades, knowledge on the pathogenesis mechanism and virulence attributes of the *Candida* species, in particular *C. albicans*, has increased tremendously. As for the host cells, host responses to microbial pathogens are among the most well-studied aspects of cellular responses to foreign agent onslaught. Pathogens usually induce phenotypic changes in host cells which are associated with significant changes in gene expression. The advent of the genomic and proteomic era has resulted in a spike in studies and publications that attempt to answer questions on these fundamental biological aspects of the infections through the use of microarray, oligonucleotide chips, bead array, RNA sequencing and other platforms. Through these new technologies, a large body of data have emerged and shown that host cells undergo extensive reprogramming of their transcriptome during infection, implying that transcriptional regulation could be a major determinant of host defence. This talk will summarize the recent hallmark publications in the field of candidiasis research, as well as briefly describe the findings in our laboratory. Using an in vivo murine model, and also in vitro human umbilical cord vein cells (HUVEC) model, we attempt to dissect the global transcriptional response to Candida infections at different infection densities. Through these endeavours, we demonstrated and identified gene expression patterns that might underlie clinical candidiasis.

## Oral-17

### The role of immune-modulating agents for severe influenza

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Pneumonia and respiratory failure are the major complications of influenza especially when there is delay in initiation of antiviral therapy. Cytokine dysregulation has been observed in patients with severe influenza such as influenza A (H5N1 and H7N9) and influenza A (H1N1)pdm09 infections but the role of immune-modulating agents is unclear due to lack of randomized controlled trial data. Prospective observational studies have shown that systemic corticosteroids may increase the risk of morbidity (eg. secondary infections) and mortality in (H1N1)pdm09 infections. Convalescent plasma appeared useful as an adjunctive therapy for treatment of influenza A (H5N1) and H1N1pdm09. The efficacy of agents with potential immunomodulating effects [eg. intravenous gammaglobulin, N-Acetylcysteine, acute use of statins, macrolides, peroxisome proliferator-activated receptors (PPAR) agonists, celecoxib, mesalazine], traditional Chinese medicine, and the role of plasmapheresis and hemoperfusion as rescue therapy would deserve more investigation and where feasible, studies by randomized controlled trials.

References:
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# Pan-Asian Biomedical Sciences Conference - Posters

| *Abstract No.* | *Title of Presentation* |
|---|---|
| P-01 | A comparison of a hold relax incorporating straight movement and incorporating PNF-components on hamstring flexibility<br>W. Kamruecha, B. Seepim, P. Boonprom, S. Pakarato |
| P-02 | Skeletal malformation of fetuses from pregnant *Sprague Dawley* rats fed *Jatropha curcas* crude oil (JCO)<br>N.H. Abdul Mutalib, Y.T. Samat, S. Sukardi |
| P-03 | Toxicity mechanism of triphenylstanum(IV) butylphenyldithiocarbamate on acute lymphooblastic cells, Jurkat E6.1<br>N. Awang, N.S.A. Kadir, N.F. Kamaludin, K.M. Chan |
| P-04 | Pegylated recombinant human arginase as a potential alternative treatment for tyrosine kinase inhibitor resistant non-small cell lung cancer<br>C.S. Lam, K.P. U |
| P-05 | Inhibition of monocytosis and oxidative stress in chronic myelomonocytic leukemia: Treatment with curcumin and epigallocatechin-3-gallate (EGCG)<br>E.S. Latif, N.K. Bohari, A.S.W. Ngui, Z. Abd Hamid, A. Hamid |
| P-06 | Prognostic association between MHC class I down-regulation relevance to tapasin promoter hypermethylation in cervical cancer development<br>S. Wanram, P. Panomket, P. Jearanaikoon, T. Limpaiboon |
| P-07 | Association between waist circumference, waist to hip ratio and body mass index with LDL-C level in persons with risk of coronary artery disease<br>P. Malila, W. Chanpho, S. Chaiyasen, W. Khamlao, W. Eungpinichpong |
| P-08 | Blood pressure responses after exercise with wand in subjects with borderline hypertension<br>P. Peungsuwan, R. Chanavirut, L. Mato |
| P-09 | Superoxide dismutase 3 (*SOD3*) gene polymorphisms and metabolic syndrome in Thai population<br>C. Settasatian, N. Decharatchakul, N. Settasatian, P. Yongsakulchai, I. Sarutipaiboon, R. Rattanatham, P. Pitivejthurakit, S. Khaichaiyaphum, P. Boonsiri |
| P-10 | The relationship of smoking related inflammatory and oxidative stress biomarkers and cardiovascular disease risk factors<br>N. Settasatian, N. Decharatchakul, C. Settasatian, P. Yongsakulchai, I. Sarutipaiboon, R. Rattanatham, S. Khaichaiyaphum, P. Pitivejthurakit, P. Boonsiri |

| *Abstract No.* | *Title of Presentation* |
|---|---|
| P-11 | A large cohort of $\beta^{+}$-thalassemia in Thailand: Molecular, hematological and diagnostic considerations<br>S. Yamsri, K. Singha, T. Prajantasen, W. Taweenan, G. Fucharoen, K. Sanchaisuriya, S. Fucharoen |
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## P-01

### A comparison of a hold relax incorporating straight movement and incorporating PNF-components on hamstring flexibility

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Previous studies suggest that hold relax (HR) technique produce greater increase hamstring flexibility than static or ballistic stretching methods. HR technique has also incorporated with straight movement and with proprioceptive neuromuscular facilitation (PNF) components, but no studies have compared the effect on hamstring flexibility between them. The objective of this study was to compare the effectiveness of a hold relax incorporating straight movement and incorporating PNF-components on hamstring flexibility. Ten male and fifty female subjects with perceived hamstring tightness and knee flexion angle between 15-70 degrees were randomly allocated to a hold relax incorporating straight movement group (n=20) and a hold relax incorporating PNF-components group (n=20) and a control group receiving no intervention. Knee flexion angle measured by passive knee extension (PKE) was measured at baseline and again following the intervention. The results showed that the mean change in knee flexion of hold relax incorporating straight movement and incorporating PNF-components were 6.49° (95% confidence interval 3.88-9.09°) and 4.32° (95% confidence interval 1.71-6.92°), respectively and significant greater than that of the control group. However, there were no significant differences when the HR groups were compared. This study concluded that a hold relax incorporating straight movement and incorporating PNF-components result in a significant increase in hamstring flexibility. However, this study only investigated the immediate effects of both HR regimes. The clinical implications of the findings are discussed.

## P-02

**Skeletal malformation of fetuses from pregnant *Sprague Dawley* rats fed *Jatropha curcas* crude oil (JCO)**

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Studies on the teratogenicity effects of *Jatropha curcas* has been reported in pregnant rats. The seeds of this plant contain curcin which is very toxic. This study was carried out to observe whether feeding *Jatropha curcas* seeds crude oil (JCO) to pregnant rats during early and late gestation will cause fetal skeletal malformations. A hundred sexually mature female rats were divided equally into 2 groups: early and late gestation. Each group was then subdivided equally into 5 groups: positive control (fed retinyl palmitate), vehicle control (corn oil), low dose (0.175ml/kg), medium dose (0.35ml/kg) and high dose (0.7ml/kg) of JCO. Rats were mated overnight and positive pregnant rats were treated accordingly on days 1-7 (early group) and days 8-14 (late group). Rats were sacrificed on day 21 of pregnancy. Fetuses were collected, weighed, processed and stained with Alizarin red. Determination of skeletal malformations was conducted using Dino Capture Microscope. Data collected showed that fetuses in the high treatment group are significantly lighter and smaller as compared to other groups. Some skeletal abnormalities in fetuses from all treated groups with the oil were observe suggesting that fetotoxic effects is apparent if dams are fed JCO during the early and late gestation periods.

## P-03

### Toxicity mechanism of triphenylstanum(IV) butylphenyldithiocarbamate on acute lymphoblastic cells, JURKAT E6.1

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Organostanum(IV) derivatives have emerged as potential metallopharmaceuticals due to their efficacy to induce cytotoxicity in various types of cancerous cell lines. Triphenylstanum(IV) butylphenyldithiocarbamate (TFBF), a novel compound has been shown to exhibit primary apoptosis in Jurkat E6.1 cells with the IC50 of 0.4 µM. In this study, the role of reactive oxygen species (ROS) and loss of mitochondrial membrane potential ($\Delta\psi m$) in Jurkat E6.1 cells is assessed along with activation of caspase 3. The measurement of ROS and loss $\Delta\psi m$ was conducted using dihydroethidium (HE) staining assay and tetramethylrhodamine ethyl ester (TMRE) staining assay, respectively. The cells were treated in the time series from ½ hour up to 4 hours prior to flow cytometric quantification. Caspase 3 activation from 1 hour up to 6 hours were evaluated using Caspase 3-Glo Luminescence Kit. As a result, there was an early increase of positive-HE stained cells as early as 1 hour of treatment as compared to negative control. Increased level of ROS lead to loss of $\Delta\psi m$. Activated caspase 3 was significant at 4 hours treatment. As a conclusion, triphenylstanum(IV) butylphenyldithiocarbamate induced apoptosis in Jurkat E6.1 cells via early production of ROS with loss of $\Delta\psi m$ and finally lead to caspase 3 Activation.

## P-04

### Pegylated recombinant human arginase as a potential alternative treatment for tyrosine kinase inhibitor resistant non-small cell lung cancer

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By far, lung cancer has the highest incidence and mortality rate in Hong Kong.(1) According to the American Cancer Society, over 85% of incidences belong to non-small cell lung cancers (NSCLC) and they are mostly diagnosed at an advanced stage.(2) Tyrosine kinase inhibitor (TKI) treatment is currently the first line therapy for advanced NSCLC.(3) However, most patients develop acquired resistance over time.(4) When both TKI treatment and chemotherapy fail, there is no third line alternative available today.(5) This study proposes that arginine starvation by recombinant human arginase (rhArg) may be a potential alternative treatment. The anti-tumour activity of recombinant human arginase (rhArg) has been previously proven on melanoma and hepatocellular carcinoma.(6)(7) This study proved the efficacy of rhArg on eight NSCLC cell lines, which include both TKI sensitive and resistant lines. In addition, the expression of ASS1, ASL and OTC genes are required for arginine synthesis in cells. Thus, expressions of these genes determine tumour sensitivity to arginase treatment. If only the cell lacks expression of any one of these three genes, rhArg depletes arginine and induces cell cycle arrest and apoptosis. This study screens for the transcriptional and translational products of the genes, and matches these results with the antitumour activity of arginase. In conclusion, results of this study have reconfirmed the linkage between antitumour activity of arginase and ASS1, ASL, OTC expressions, and proved rhArg as a potential alternative to TKI resistant cancer treatment.

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## P-05

**Inhibition of monocytosis and oxidative stress in chronic myelomonocytic leukemia: Treatment with curcumin and epigallocatechin-3-gallate (EGCG)**

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Chronic myelomonocytic leukemia (CMML), a clonal hematopoietic stem cell disorder indirectly associated with oxidative stress, is characterized by absolute monocytosis in the peripheral blood and abundance of dysplastic cells in the bone marrow. Current clinical chemotherapy of CMML is Imatinib, which unfortunately triggers various side effects in patients. Hence, alternative chemotherapeutic agents with lesser or none side effects are warranted, preferably of the natural nutrimedicinal resources with promising anticancer properties.

Curcumin (CUR) and EGCG, compounds derived from turmeric and green tea respectively, are natural resources products proven to possess antioxidant and anticancer properties. This study aims to determine the in vivo-effects of CUR and EGCG on monocytosis and oxidative stress in CMML mice. WEHI-3 (murine CMML) cells were inoculated intraperitoneally in BALB/c mice to produce leukemia in the animal model. Mice were groups into: (1) normal, (2) leukemia untreated or treated with Imatinib (25mg/kg bw), (3) leukemia treated with CUR (40mg/kg bw) or EGCG (20mg/kg bw) or combination of both compounds. Treatments were given intensively for 14 consecutive days.

Results of hematological test show that CUR and EGCG are capable in reducing the average of total white blood cells, blasts, monocytes and neutrophils in peripheral blood of leukemic mice. Infiltrations of leukemic cells in spleen and liver have also become lesser with CUR and EGCG treatment. Compared to those without treatment, protein damage and malondialdehyde (MDA) levels are also significantly lower in these organs. These reductions are accompanied by significantly higher levels of antioxidant glutathione (GSH), activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes, induced by CUR and EGCG treatment. Conclusively, CUR and EGCG efficiently inhibit proliferation of leukemic cells, thus reducing monocytosis and oxidative stress, more effectively with the combination of both CUR and EGCG. The outcome of this study reflects the potential of CUR and EGCG in becoming alternative treatment for CMML.

## P-06

### Prognostic association between MHC class I down-regulation relevance to Tapasin promoter hypermethylation in cervical cancer development

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**Problem**: MHC class I down-regulation associated with antigen processing machinery defect is proposed as a crucial event for cervical cancer (CXCA) evasion from host immune response but the exact mechanism is unknown. We hypothesized whether aberrant Tapasin DNA methylation mechanism related to MHC class I down-regulation as well as pathogenic viral crucial factors and clinical outcome of progression.

**Objective**: To determine Tapasin promoter hypermethylation and MHC class I expression with their association with clinical outcome of progression.

**Strategy**: A cohort study, MS-PCR and quantitative methylation–specific PCR (QMSP) of Tapasin was performed using methylation-sensitive high-resolution melting curve analysis (MS-HRM). Embedded paraffin samples were examined for protein expression of MHC class I and Tapasin using immuno-histochemical staining. High risk (HR) HPV16 genotype was identified by E6/E7 nested multiplex PCR. The HPV16 physical state was determined using quantitative PCR via TaqMan probe assay, indicating for HPV16 E2 and E6 ratio interpretation. Association study between MHC class I expression and Tapasin promoter hypermethylation as well as clinical outcome of progression were investigated relevance to HR-HPV crucial factors.

**Significance**: MS-PCR results showed percentage of Tapasin promoter hypermethylation at 19.04% (8/42 cases) and 54.34% (25/46 cases) of pre-invasive lesion and invasive CXCA, respectively. MS-HRM showed increase percentage of Tapasin promoter hypermethylation at 19.04% (8/42 cases) and 54.34% (25/46 cases) of pre-invasive and invasive CXCA, respectively. Interestingly, the combination study between MHC class I and Tapasin down-regulation and HPV16 physical status were discovered. We found MHC class I loss of expression associated with Tapasin promoter hypermethylation value for integrated HPV16 occurrence as well as progression result during cervical cancer development. Our results recommend that MHC class I down-regulation associated with Tapasin promoter hypermethylation mechanism. The combination of HPV16 physical status and MHC class I with Tapasin expression should be used for prognostic markers to predict clinical outcome of progression during cervical carcinogenesis. We proposed that host immune responses according to down-regulation of MHC class I associated with Tapasin promoter hypermethylation related to clinical outcome of progression. The epigenetic study and their relevance to down-regulation of MHC class I with other antigen processing machinery mechanisms especially how to be results of progression should be further investigated.

## P-07

### Association between waist circumference, waist to hip ratio and body mass index with LDL-C level in persons with risk of coronary artery disease

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Coronary artery disease is increased every year according to the report from the ministry of public health. Thus, it is considered a major health problem of the country. The objective of this study was to determine the association between waist circumference, waist to hip ratio and body mass index with the level of low density lipoprotein (LDL-C) in the blood. Volunteers who attended a project for reduce risk factors of coronary artery disease at a subdistric in Khon Kaen province participate in the study. Data were obtained from 102 adults between 45 to 85 years of age (31 males, 71 females). The volunteers were assessed for plasma LDL-C, waist circumference, waist to hip ratio and body mass index. The results showed that the waist circumference and waist to hip ratio had association with the LDL-C (OR= 1.64, 95% CI=0.50 to 3.33; $p < 0.001$ and OR= 0.66, 95% CI=0.28 to 1.56; $p < 0.001$). Conclusion we found this correlation that might be predicted risk cardiovascular for cardiovascular disease and these data provide potential clinical health status in local area in Khon Kaen, may be useful for prevention and health promotion strategies in Thailand.

## P-08

### Blood pressure responses after exercise with wand in subjects with borderline hypertension

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**Introduction**: Epidemiologic studies indicate that uncontrolled increased blood pressure leads to stroke, coronary heart disease, and congestive heart failure. Effect of aerobic exercise is addressed in several studies that can reduce the blood pressure (BP) in hypertensive patients. Thai exercise with wand is a simple form that may be able to reduce the BP in hypertensive patients.

**Methods**: The study design was cross-over trial. Twenty-six patients with borderline hypertension (aged 57 ± 7 years, 17 female) were allocated into a control (CC, rest in sitting) or exercise with wand (EC) conditions, duration of the CC and the EC was 30-min, each intervention was separately a 1-wk. They were measured blood pressure (Sphygmomanometer) in a sitting position before and after the experiment immediately, and every 10 min until to 120 min.

**Results**: Comparing between conditions, systolic blood pressure (SBP) and mean arterial pressure (MAP) significantly reduced 3.8 mm Hg ($p$-value< 0.001) and 2.6 mm Hg ($p$-value< 0.01) for the first 30-90 min after EC, respectively. In CC didn't found changing BP.

**Conclusions**: The reduction of blood pressure after EC was short effect. We suggest that if hypertensive patients regularly perform it may be more benefit the resting. Thus, long-term effect of EC training on BP should be considered in the future study.

## P-09

### Superoxide dismutase 3 (*SOD3*) gene polymorphisms and metabolic syndrome in Thai population

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**Introduction:** Superoxide dismutase 3 (SOD3) is an antioxidant enzyme that catalyzes the dismutation of superoxide radicals into hydrogen peroxide and oxygen. This enzyme is anchored to the extracellular matrix and cell surface. Major sources of SOD3 are blood vessels, lung, and heart. Two *SOD3* gene polymorphisms have been studied in other ethnic groups. The *SOD3* polymorphism, rs2536512, is a dimorphism of *G* to *A* alteration that leads to the change of amino acid position 58 (*Ala58Thr*) and has been associated with diabetes and hypertension, the components of metabolic syndrome (MetS). Other *SOD3* gene polymorphism, rs2855262 (*Ex3-489T*>C) is located on 3'untranslated region of the gene and have been utilized in *SOD3* haplotype analysis. However the association between these polymorphisms and metabolic syndrome (MetS) has remained undetermined in Thais. The objective of this study was therefore to investigate the association of *SOD3* gene polymorphisms (rs2536512 and rs2855262) with the risk of MetS in Thai population.

**Method:** A total of 397 community-based Thai subjects (mean age: 51.6±10.1 years; 242 males and 155 females) were recruited. The prevalence of MetS was determined according to modified National Cholesterol Education Program III (modified NCEP) criteria. *SOD3* gene polymorphisms (rs2536512 and rs2855262) were determined in genomic DNA using allele specific polymerase chain reaction (AS-PCR) technique.

**Result:** The prevalence of MetS in studied population was 31%. For rs2536512, the respective frequencies of *G* and *A* alleles were 58.8% and 41.2%, whereas the frequencies of *T* and *C* alleles for rs2855262 were 53.5% and 46.5%. For each individual *SOD3* polymorphism, there was no association with the risk of MetS and no significant association with lipid parameters, fasting blood sugar (FBS), systolic blood pressure (SBP) and diastolic blood pressure (DBP) between genotype groups. However, with the combination between two dimorphisms of *SOD3*, the combined *GG* with *TT* was associated with the increase risk of MetS (OR = 2.18; 95% CI 1.03-4.59) as compared with subjects who carried combined genotypes *AA* + *CC*. The *GG* and *TT* subjects also had higher levels of FBS and SBP than the *AA* and *CC* subjects (p=0.018 and p=0.049, respectively). Strong (D'=0.85) linkage disequilibrium (LD) between these two studied polymorphisms was also observed.

**Conclusion:** The present study demonstrated the association of combined two *SOD3* gene polymorphisms with the risk of MetS in Thai populations. The combined *GG* with *TT* had also associated with high levels of FBS and SBP that increased the risk of MetS.

## P-10

### The relationship of smoking related inflammatory and oxidative stress biomarkers and cardiovascular disease risk factors

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**Background:** Cardiovascular disease (CVD) is among the diseases with high mortality rate. In Thai population, as of 2011, the mortality rate was 343 and 280 cases per 100,000 population in male and female, respectively. Major CVD risk factors include tobacco smoking, dyslipidemia, hypertension, obesity, and diabetes mellitus. Oxidative stress and inflammatory condition are the results of risk factor-related pathophysiology of CVD. The combinations of these risk factors may increase the incidence and severity of CVD. High-sensitive C-reactive protein (*hs-CRP*) is an inflammatory biomarker, whereas blood malondialdehyde (MDA) represents an oxidative stress biomarker for CVD risk prediction. The aim of the present study was to evaluate the relationship between blood levels of these biomarkers, hs-CRP and MDA, and the combination effect of CVD risk factors, by comparing the level of these biomarkers between studied groups according to the number of combination CVD risk factors.

**Methods:** A total of 342 Thai subjects were recruited and divided into 6 groups. The first three groups were never-smokers and the second three groups were current-smokers. Each set of three groups consist of those without CVD risk factor, those with one to two risk factors, and those with three or more risk factors. Blood hs-CRP and MDA were respectively determined by immunonephelometric and thiobarbituric acid method.

**Results:** In this study, dyslipidemia was the most common CVD risk factor (91.8%) followed by obesity (33.3%), hypertension (23.4%), and diabetes mellitus (5%). Of all subjects, 2.3% and 3.2% respectively were never- and current-smokers without CVD risk factors. Never- and current-smokers with one to two CVD risk factors constituted 42.4% and 38.6% of all subjects whereas those with more than two CVD risk factors were 9.1% and 4.4%, respectively. The lowest mean levels of hs-CRP and MDA (1.1±1.3 mg/L and 3.8±0.5 μmol/L, respectively) were observed in never-smokers without CVD risk factors, while current-smokers with more than two risk factors revealed the highest levels of these biomarkers (11.1±32.4 mg/L and 6.7±2.3 μmol/L). The tendency of increasing mean levels of MDA was significantly demonstrated according to the increasing number of combined CVD risk factors and smoking status ($p < 0.001$ with p-trend $< 0.001$). The increasing levels of hs-CRP were observed from those without CVD risk factors to those with more than two risk factors in both never-smokers (1.1±1.3 mg/L vs 2.9±3.7 mg/L) and current-smokers (1.4±2.5 mg/L vs 11.1±32.4 mg/L), $p=0.003$.

**Conclusion:** The present study demonstrated the effect of smoking and combined CVD risk factors on the increasing level of oxidative stress and inflammatory markers, both of which are pathophysiologic conditions related to CVD.

## P-11

### A large cohort of $\beta^+$-thalassemia in Thailand: Molecular, hematological and diagnostic considerations

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β-Thalassemia is a group of genetic disorders resulting from the defects of β-globin chain production. Two main types, $\beta^0$- and $\beta^+$-thalassemia, are defined based on the absence or reduction of β-globin chain systhesis, respectively. Most of the mild cases were associated with $\beta^+$-thalassemia genes. Couple at risk of having fetus with a mild $\beta^+$-thalassemia disease should be avoided from an unnecessary obstetrical risk of miscarriage associated with prenatal testing procedures. Identification and differentiation of β-thalassemia mutations are therefore essential for planning of appropriate management, genetic counseling and prediction of the clinical outcome. We report the molecular and hematological characteristics associated with $\beta^+$-thalassemia in Thailand. Study was done on a cohort of 21,068 unrelated subjects whose blood specimens were referred to our center at Khon Kaen University in northeast Thailand for hemoglobinopathies investigation during January 2010 to June 2014. Hematological parameters were recorded and diagnosis was done using Hb and DNA analyses. Among 21,068 subjects, 2,637 (12.5%) were found to carry β-thalassemia. Of these 2,637 cases, 705 (26.7%) carried $\beta^+$-thalassemia with eight different mutations including 6 promoter mutations; NT-28(A-G), NT-31(A-G), NT-50 (G-A), NT-86 (C-G), NT-87 (C-A) and NT-90 (C-T) and two β-thalassemic hemoglobinopathies; Hb Malay (codon 19; A*A*C-A*G*C) and Hb Dhonburi (codon 126; G*T*G-G*G*G). Hematological features of carriers with these $\beta^+$-thalassemia (n=528) were compared with those with $\beta^0$-thalassemia (n=309). As for subjects with Hb E-$\beta^+$-thalassemia (n=177) whose data were presented along with Hb E-$\beta^0$-thalassemia in our series (n=94). All patients with Hb E-$\beta^+$-thalassemia were associated with mild thalassemia intermedia phenotypes. Although most of the carriers of these $\beta^+$-thalassemia had elevated Hb $A_2$ and mild hypochromic microcytosis, some demonstrated borderline MCV and MCH values which could complicate carrier screening. Analysis of α/ β-globin mRNA ratio in representative cases with normal, Hb E trait, $\beta^+$-thalassemia trait, Hb Dhonburi trait and $\beta^0$-thalassemia trait demonstrated the average values of 1.1, 1.7, 2.1, 1.7 and 3.1, respectively which is helpful in identification and differentiation of the cases. These results provide useful information for carrier screening and genetic counseling in a prevention and control program of thalassemia in the region.

## P-12

### Activation of G protein-coupled estrogen receptor (GPER) inhibits P2Y receptor-mediated $Ca^{2+}$ signalling and cytokine secretion via cAMP-dependent PKA pathway in human bronchial epithelia

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P2Y receptor activation by UTP and UDP causes the release of inflammatory cytokines in the bronchial epithelium. In addition to the classical nuclear hormone receptors ERα and ERβ, a novel estrogen (E2) receptor, G protein-coupled estrogen receptor (GPER), was recently identified. Our study aimed to investigate the cellular mechanisms underlying the inhibitory effect of GPER or E2 receptor activation on P2Y receptor-mediated $Ca^{2+}$ signalling pathway and cytokine production in human bronchial epithelia.

Both human bronchial epithelial cell line 16HBE14o- and primary human bronchial epithelial (HBE) cells (ScienCell Research Laboratories, San Diego, CA, USA) were used. Intracellular calcium ($[Ca^{2+}]_i$) increase was monitored by calcium imaging technique using Fura-2. Real-time cAMP change was recorded using CFP-Epac-YFP FRET reporter. Cytokine concentration in the culture medium was measured by ELISA.

Stimulation of primary HBE or 16HBE14o- cells with E2 or with the specific agonist of GPER, G1, rapidly attenuated a UDP- or UTP-evoked increase in $[Ca^{2+}]_i$ while this effect was reversed by GPER specific antagonist, G15. E2 or G1 inhibited the secretion of two pro-inflammatory cytokines, interleukin (IL) - 6 or IL-8, in cells stimulated by UDP and UTP. The cytokine release was reduced in the presence of an intracellular $Ca^{2+}$ chelator, BAPTA-AM. Both E2 and G1 stimulated a real-time increase in cAMP level in 16HBE14o- cells, which could be inhibited by adenylate cyclase inhibitor, MDL 12330A or SQ 22536. The inhibitory effect of E2 or G1 on P2Y receptor-induced $Ca^{2+}$ increase was reversed by treating the 16HBE14o- cells with a protein kinase A (PKA) inhibitor, H89.

Our data demonstrate that the inhibitory effect of G1 or E2 on P2Y receptor-mediated $Ca^{2+}$ mobilization and cytokine secretion was possibly due to the activation of cAMP-dependent PKA pathway by GPER in human bronchial epithelia.

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## P-13

### Regulation of inflammation in the Central Nervous System: Platelets as sensors of neuronal damage

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Inflammation in the central nervous system (CNS) is a complex process with a high social and economic impact worldwide. Currently there is no effective therapy for prevention and treatment of CNS inflammation that accompany many neurodegenerative diseases such as multiple sclerosis (MS) or Alzheimer's disease. Our study is focused on regulation of inflammation in the CNS by platelets. Platelets respond to a vascular damage, but their role in the neurodegenerative diseases is not well known. We found that administration of brain lipid rafts induced a massive platelet activation and degranulation resulting in anaphylaxis in mice. Platelets reacted with sialated gangliosides integrated in lipid rafts of astrocytes and neurons. The brain-specific gangliosides GT1b and GQ1b were recognized by the platelets and this recognition occurred during disruption of blood brain barrier. During neuroinflammation, platelets accumulated in the CNS and secreted proinflammatory factors such as IL-1, PAF and serotonin (5-HT). Further implications of pathogenic and regulatory roles of platelets and their direct interactions with CD4 T cells in MS and traumatic brain injury will be further discussed. Thus the study determines a new role of platelets as "innate immune cells" that directly recognize a neuronal damage and contribute to inflammation in the CNS.

## P-14

### Anti-inflammatory effect of *Clarias Batrachus* fish oil on LPS-induced raw 264.7 cells

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Omega 3 and 6 are polyunsaturated fatty acids commonly found in fish oils. Previous study had demonstrated that *Clarias batrachus*, a commonly found freshwater in Asia, contain high levels of Omega 3, 6 and 9 PUFAs among other freshwater fish. PUFA has the ability to reduce the production of inflammatory eicosanoids, cytokines and reactive oxygen species (ROS). In this study, the anti-inflammatory effects of *Clarias batrachus* fish oil were investigated using LPS-treated RAW 264.7 cells as a model. Our data demonstrated that fish oil induce no cytotoxic effects against macrophage RAW 264.7 cells as determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. The level of glutathione as assessed by Ellman method showed significant different in GSH level on RAW 264.7 cell treated with different concentration of *Clarias batrachus* fish oil. The expression of inflammatory protein COX-2 on LPS-treated cells for 24 hours as determined using the immunoblot was higher as compared to cells undergone pre-treatment of *Clarias batrachus* fish oil prior to LPS exposure. Additionally, *Clarias batrachus* fish oil also has the ability to increase GSH concentration which may contribute to the non-cytotoxic effect shown using the MTT assay. In conclusion, *Clarias batrachus* fish oil does not cause any cytotoxic effect on RAW 264.7 cells and abled to reduce ROS via increasing the level of GSH and reducing the expression of COX-2. Hence, Clarias batrachus fish oil has the potential to be developed as anti-inflammatory with antioxidant properties.

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## P-15

### *In vitro* antioxidant activity and immune cell safety of Thai rat-tailed radish extract

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Thai rat-tailed radish (TRT) or *Raphanus sativus L. var. caudatus Alef* belongs to the family Brassicaceae or Cruciferae. It is commonly found in Northern Thailand and is eaten as a side dish or cooked. Brassicaceae are popular vegetables globally and their consumption reduces the risk for many types of cancer. This study aimed to demonstrate an antioxidant activity potential and safety on immune cell of TRT to substantiate the value. The edible parts (stem and pod) from TRT were tested for *in vitro* antioxidant activity and cell viability by FRAP assay and MTT assay, respectively. TRT at 5, 50 µg/ml was found to have an antioxidant activity (0.004-0.051 µmol Trolox equivalent). The stem sample exhibited higher reducing power (about 1.4 fold) when compared to pod sample. However, non-significant of the percentage of immune cell viability was found between the stem and pod parts (89% vs 87%). These results suggested that TRT have and antioxidant potential and safety for immune cell viability. Further study is necessary for isolation and characterization of the active antioxidant agents, which can be used to treat various oxidative stress-related diseases.

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## P-16

### Relapsed melioidosis caused by biofilm mutant in C57BL/6 mice

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*Burkholderia pseudomallei* are causative agents of melioidosis, a disease that has variable clinical manifestations, such as asymptomatic chronic inflammation, acute septicemia, and septic shock. It has a high rate of relapse and the mechanism of this is still unclear. The objective of this study was to establish relapsed infection of C57BL/6 mice by induction with *B. pseudomallei*. Low doses of *B. pseudomallei* H777 and its biofilm defective mutant (M10) were intra-gastrically fed to C57BL/6 mice. All the infected mice had suppressed immune status by intra-peritoneal injection of hydrocortisone at 2.5 mg per mouse at day 60 post-infection. Inflammatory response to the infection was investigated by histo-pathological studies and monitored bacterial count in blood and organs. All the infected mice were found to have a high infiltration of mononuclear cells at day 60 post-infection. The results showed high bacteria counts in blood in both strains post suppressed immune status two days. Biofilm mutant and wild type strains can produce relapse in C57BL/6 mice but the latter is responsible for significantly more severe inflammation than biofilm mutant. Thus, low immune status may cause relapsed melioidosis in hosts with chronic inflammation.

## P-17

### Replication of avian influenza A virus strain H5N1 in madin-darby canine kidney cells

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Traditional influenza vaccine production in chicken embryonated eggs introduces hazardous microbiological materials in the vaccines. Hence, the use of cell culture system such as Madin-Darby Canine Kidney (MDCK) cell which supports efficient growth of influenza virus is much advocated presently. It is essential to understand virus replication patterns in MDCK cells prior to utilizing it in influenza vaccine production. Firstly, MDCK cells were infected with $10^{-2}$ to $10^{-12}$ avian influenza virus H5N1 (AIV H5N1) (5858/2004) dilutions for 24 h in order to determine its $TCID_{50}$ (50% tissue culture infectious dose). The virus dilution causing 50% cytopathic effects (CPE) was used as $TCID_{50}$ to infect 80-100% confluently grown MDCK cells for 0-48 h. The CPE was observed and cell death was determined at 2-h time intervals. The virus-infected cells and media were collected for virus RNA analysis. The extracted total RNA was used to form the first-strand cDNA which served as template in the M2 gene amplification. The PCR product was analyzed and quantified on 1% (w/v) agarose gel. The results showed that the $TCID_{50}$ of AIV H5N1 in MDCK cells was $10^{-9}$ dilution. CPE was observed after 2-h post-infection and the maximal CPE was achieved at 48 h. The cell death percentage in virus infected cells showed a strong and positive correlation with the infection period ($r = 1.0$, $n = 9$, $p < 0.01$). The amount of M2 gene amplified from the infected media ($r = 0.471$, $n = 9$, $p > 0.05$) and infected cell ($r = 0.73$, $n = 9$, $p < 0.05$) was positively correlated with the infection period. Collectively, this implies that the virus replication increases with an incline in viral replication period. Although CPE was observed as early as 2-h post infection, the M2 gene was only amplified from the infected media and cells after 48 h and 24 h, respectively. This signifies that the AIV H5N1 is pathogenic and able to cause cytopathology in host cells even at low virus load. In conclusion, this study has demonstrated that MDCK cell is a suitable system to support AIV H5N1 growth and thereby suitable to be used for influenza vaccine production.

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## P-18

### Anti-leptospiral potential of *Zingiber zerumbet* (L.) Smith Crude extracts against *Leptospira spp.*

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Leptospirosis is one of the emerging infectious diseases that the number of cases had been increased progressively in Malaysia. Numerous attempts have been made to control the disease by using chemoprophylaxis but only showed limited success. The present study investigates the anti-leptospiral potential of *Zingiber zerumbet* crude extracts which are hexane, ethyl acetate and methanol. The extracts were assayed for anti-leptospiral activity using broth microdilution method towards *Leptospira interrogans* (serovar Batavie, Canicola, Australis) and *Leptospira biflexa* serovar Patoc. On the other hand, DNA damaging properties of *Z. zerumbet* on DNA of *Leptospira sp.* was done by incubating the DNA of *Leptospira sp.* in the presence or absence of *Z. zerumbet* crude extracts and analyzed by electrophoresis. The anti-leptospiral activity of *Z. zerumbet* hexane extracts gave $IC_{50}$value at 248 µg/ml towards *L. interrogans* serovar Canicola, 125 µg/ml towards *L. interrogans* serovar Australis, and 109 µg/ml towards *L. biflexa* serovar Patoc. However, ethyl acetate and methanol extracts, did not show much of anti-leptospiral activity. Since the hexane extract of *Z. zingiber* had anti-leptospiral activity, the DNA damaging properties of this extract was tested according to their $IC_{50}$ and $IC_{25}$ value that was specific to each serovars. The results showed that there were DNA damage as proven by the appearance of fragmented DNA on the gel for *L. biflexa* serovar Patoc and *L. interrogans* serovar Australis. However, there were no sign of DNA damaging activities towards *L. interrogans* serovar Canicola and Bataviae. In conclusion, *Z. zerumbet* hexane extracts can inhibit the growth and damage the DNA of *Leptospira spp*., further studies need to be done to evaluate for anti-leptospiral activity of *Z. zerumbet* hexane extracts.

## P-19

### The association of Tamm-Horsfall protein with the levels of potassium, magnesium and calcium in the urine of stone formers and healthy subjects

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**Introduction:** Tamm-Horsfall protein (THP) is known as urinary glycoprotein that affects the formation of calcium-containing kidney stones. THP inhibits the aggregation of calcium oxalate crystals and provides a defense against urinary tract infection. Thus, decreased level of THP may affect to stone formation and/or urinary tract infection. Since urinary tract infection may have the effect on kidney stone formation, to address whether THP itself has the effect on stone formation. However, previous studies on the association between THP and various urinary parameters had not considered bacteriuria factor. Therefore, we examined the association of THP with the levels of $K^+$, $Mg^{2+}$ and $Ca^{2+}$ in the urine of stone formers and healthy subjects with negative urine culture.
**Methods:** Thirty male subjects without bacteriuria were included in this study (15 subjects with calcium stone former and 15 healthy subjects in an age-matched comparison group). Bacteriuria screening was done by standard urine culture method. The stone composition was analyzed by infrared spectrophotometry. Urinary $K^+$, $Mg^{2+}$ and $Ca^{2+}$ levels were determined by atomic absorption spectophotometer. Urinary THP level was evaluated by using enzyme linked immunosorbent assay.
**Results:** In comparison with the healthy control group, urinary $Ca^{2+}$/creatinine ratio was significant lower in the stone former group (1.51±0.58 vs 0.54±0.08, $P<0.05$), whereas the urinary $K^+$/creatinine ratio (12.79±3.17 vs 9.50±1.10), urinary $Mg^{2+}$/creatinine ratio (1.10±0.38 vs 0.58±0.07) and urinary THP /creatinine ratio (2.28±0.86 vs 1.63±0.29) were not significantly different. Using linear regression analysis, the healthy control group clearly showed positive correlation between THP/creatinine and $Ca^{2+}$/creatinine ratios suggesting THP as a protective stone formation.
**Conclusions:** These findings support the evidence that THP may associate with the pathogenesis of calcium stone formation in the male kidney stone formers.
**Key words:** Tamm-Horsfall protein, kidney stone, bacteriuria, calcium

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## P-20

### Palm oil tocotrienol-rich fraction attenuates testicular oxidative damage induced by fenitrothion

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Exposure to organophosphates insecticides including fenitrothion (FNT) has been reported to cause testicular oxidative damage. Palm oil tocotrienol-rich fraction (TRF) is a well-known antioxidant that has shown potential in reducing oxidative stress in various pathological conditions. Thus, the present study was conducted to determine whether TRF could prevent the testicular damage induced by FNT. Mature male Sprague-Dawley rats (n=40) were given FNT (20 mg/kg), TRF (200 mg/kg) and TRF+FNT daily via gavage for 28 consecutive days. Parameters evaluated included oxidative stress status, heat shock protein-70 (HSP70) expression and germ cell apoptosis. Co-administration of TRF in FNT-treated rats increased the activities of enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione s-transferase ($p<0.05$). TRF also increased ferric reducing antioxidant power (FRAP) as well as levels of non-enzymatic antioxidants such as glutathione, compared with the FNT group alone ($p<0.05$). TRF reduced lipid peroxidation and protein oxidation by significantly lowering the malondialdehyde and protein carbonyl levels in FNT-treated rats ($p<0.01$). HSP70 expression and apoptotic germ cell count in the testis was significantly reduced in the TRF+FNT group ($p<0.05$). In conclusion, TRF was able to partially alleviate testicular damage in FNT-intoxicated rats.

## P-21

### Comparative myelotoxicity effect of hydroquinone (HQ) on hematopoietic progenitors and stem cells

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Benzene exposure has been linked to hematotoxicity and leukemogenicity. The impact of benzene exposure on complex microenvironment of Hematopoetic Stem Cells (HSCs) niche, comprising of HSCs and lineage-specific progenitors remains elusive. Thus, study on benzene-targeting HSCs niche could establish potential mechanism linking benzene to the alteration in HSCs niche. This study aims to evaluate toxic effect of a benzene metabolite, Hydroquinone (HQ) on HSCs and to compare its myelotoxicity effect on different types of myeloid-committed progenitors. HQ was added at varying concentrations (0 – 50 µM) for 24 hours to the murine bone marrow cells (BMCs) cultures. Viability of HQ-treated BMCs were determined by using 3-(4,5-dimetylthiazol-2-yl)- 2,5-diphenyltetrazoliumbromide (MTT) assay. Expression of cellular surface antigen for HSCs (Sca-1) was confirmed by flow cytometer. Myelotoxicity of HQ was studied using the colony-forming unit (CFU) assay for the following myeloid progenitors: CFU-granulocyte/erythrocyte/macrophage/ megakaryocyte (CFU-GEMM), CFU-granulocyte/macrophage (CFU-GM), CFU-granulocyte (CFU-G), CFU-macrophage (CFU-M), CFU-erythroid (CFU-E) and Burst-forming unit erythroid (BFU-E). HQ reduced ($p<0.05$) viability of BMCs at 25 µM and 50 µM, and the $IC_{10}$, $IC_{25}$, and $IC_{50}$ were 17 µM, 23 µM and 35 µM, respectively. Reduced ($p<0.05$) Sca-1 expression at 17 µM, 23 µM and 35 µM indicates cytotoxic effect of HQ on cultured mouse hematopoietic progenitors and stem cells. Myeloid clonogenic assay revealed reduced ($p<0.05$) total colony numbers in the presence of HQ at 6.25 µM and a complete inhibition in colony growth at higher concentrations (12.5 µM to 35 µM). HQ reduced ($p<0.05$) the growth of CFU-GEMM, CFU-GM and CFU-G at 6.25 µM, while the growth of CFU-M, CFU-E and BFU-E were not remarkably affected at its lower concentrations (1.56 µM and 6.25 µM). In conclusion, myelotoxicity effect of HQ could be mediated through a lineage-dependent response which may be responsible for in vivo hematological troubles. Further in vivo study is warranted to confirm this hypothesis.

## P-22

### Telomerase reactivation in Werner syndrome iPSC and prevention of premature aging at ground state

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Werner syndrome (WS) is an adult progeria characterized by accelerated aging after puberty. WS patients exhibit short stature, bird-like face, gray hair, bilateral cataract, osteoporosis, skin ulcer, hypogonadism, and high incidence of non-epithelial neoplasms. These clinical features demonstrate a severe deterioration in cells mostly of the mesenchymal origin. Genetic and biochemical studies indicate that WRN mutation contributes to the pathogenesis of premature aging. Loss of WRN protein impairs DNA replication and repair, and results in increased telomere loss in actively dividing cells such as fibroblasts. We suggest that WRN-dependent accelerated aging is lineage specific. To model premature aging, we reprogrammed WS fibroblasts to pluripotent stem cells (iPSC). Reprogrammed WS iPSCs were corrected for their telomere defect, as revealed by successful telomerase reactivation and telomere elongation. Additionally, WS iPSC were highly similar to wild-type iPSCs in gene expression and showed no significant slowdown of DNA synthesis. An explanation for this corrected cellular aging phenomenon is the prevention of telomere defect by telomerase at ground state. To understand the role of telomerase in protecting against accelerated aging, we differentiated WS iPSC to mesenchymal stem cells (MSCs). Upon differentiation, telomerase activity decreased significantly. Newly derived WS MSCs divided actively in culture. However, WS MSC entered senescence earlier than wild-type MSC and demonstrated accelerated telomere shortening and loss of sister telomeres at the lagging strand. Forced expression of hTERT or depletion of p53 rescued the accelerated senescence. Our data suggest a central role of telomerase in protecting specific lineage of stem cells from premature aging.

## P-23

**Heat shock 70kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling**

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The heat shock 70kDa protein 5 (Hspa5) also known as binding immunoglobulin protein (Bip) or glucose regulated protein 78 (Grp78), belongs to the heat shock protein 70kDa family. As a multifunctional protein, it participates in protein folding, calcium homeostasis and serves as an essential regulator of the endoplasmic reticulum (ER) stress response. It has also been implicated in signal transduction by acting as a receptor or co-receptor residing at the plasma membrane. Its function during embryonic development, however, remains largely elusive. In this study, we used morpholino antisense oligonucleotides (MO) to knockdown Hspa5 activity in *Xenopus* embryos. In Hspa5 morphants, pronephros formation was strongly inhibited with the reduction of pronephric marker genes *lhx1*, *pax2* and atp1b1. Pronephros tissue is induced *in vitro* by treating animal caps with *all-trans* retinoic acid (atRA) and activin. Depletion of Hspa5 in animal caps, however, blocked the induction of pronephros as well as reduced the expression of RA-responsive genes, suggesting that knockdown of Hspa5 attenuated RA signaling. Knockdown of Hspa5 in animal caps resulted in decreased expression of *lhx1*, a transcription factor directly regulated by RA signaling and essential for pronephros specification. Co-injection of Hspa5MO with *lhx1* mRNA partially rescues the phenotype induced by Hspa5MO. These results suggest that the RA-lhx1 signaling cascade is involved in Hspa5MO induced pronephros malformation. This study shows that Hspa5, a key regulator of the unfolded protein response, plays an essential role in pronephros formation, which is mediated in part through RA signaling during early embryonic development.

## P-24

**Modulation of calcium-induced cell death in human neural stem cells by the novel peptidylarginine deiminase-AIF pathway**

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PADs (peptidylarginine deiminases) are calcium-dependent enzymes that change protein-bound arginine to citrulline (citrullination/deimination) affecting protein conformation and function. PAD up-regulation following chick spinal cord injury has been linked to extensive tissue damage and loss of regenerative capability. Having found that human neural stem cells (hNSCs) expressed PAD2 and PAD3, we studied PAD function in these cells and investigated PAD3 as a potential target for neuroprotection by mimicking calcium-induced secondary injury responses. We show that PAD3, rather than PAD2 is a modulator of cell growth/death and that PAD activity is not associated with caspase-3-dependent cell death, but is required for AIF (apoptosis inducing factor)-mediated apoptosis. PAD inhibition prevents association of PAD3 with AIF and AIF cleavage required for its translocation to the nucleus. Finally, PAD inhibition also hinders calcium-induced cytoskeleton disassembly and association of PAD3 with vimentin, that we show to be associated also with AIF; together this suggests that PAD-dependent cytoskeleton disassembly may play a role in AIF translocation to the nucleus. This is the first study highlighting a role of PAD activity in balancing hNSC survival/death, identifying PAD3 as an important upstream regulator of calcium induced apoptosis, which could be targeted to reduce neural loss, and shedding light on the mechanisms involved.

## 11 December 2014 (Thursday) 17:20 – 18:30

## ASIA-International Biomedical Science Consortium Outstanding Poster Award Competition

*****Presenting authors please be available by your poster for answering questions*****

| *Abstract No.* | *Title of Presentation* |
|---|---|
| PS-01 | Carcinogenic liver fluke secretes exosomes that promote cholangiocytes to adopt a tumorigenic phenotype<br>S. Chaiyadet, J. Sotillo, M. Smout, C. Cantacessi, M.K. Jones, M.S. Johnson, L. Turnbull, C.B. Whitchurch, J. Mulvenna, P.J. Brindley, J.M. Bethony, T. Laha, B. Sripa, A. Loukas |
| PS-02 | Anti-senescence activity of a cancer-causing protein cyclin D1<br>N. Ketaroonrut, P. Laphanuwat , S. Jirawatnotai |
| PS-03 | LAMP-gold-nanoparticle technique as a simple and rapid detection method of HPV16<br>R. Kumvongpin, C. Wilailuckana, N. Sae-ung, P. Boonsiri , J. Daduang |
| PS-04 | Anti-apoptotic parasite's molecule, opisthorchis viverrini-thioredoxin-1 related human apoptosis signal regulating kinase-1 in cholangiocytes cell<br>P. Matchimakul, G. Rinaldi, T. Laha, V.H. Mann, A. Popratiloff, S. Suttiprapa, S. Kaewkes, P.J. Brindley, B. Sripa |
| PS-05 | Physical mapping between Y-box DNA/RNA-binding factor, YB-1truncated proteins and multivalent 11 zinc fingers transcriptional factors, CTCF and BORIS in multiforme RGBM cell lines<br>D.K. Mohd Azman, S. Shamsuddin, S.C. Tan |
| PS-06 | Uptake rate of colorectal cancer screening in Thailand and effects of user fee<br>U. Saengow, S. Birch, A. Geater, V. Chongsuvivatwong |
| PS-07 | Total serum bile acids as a potential marker for the diagnosis of cholangiocarcinoma<br>S. Sombattheera, T. Proungvitaya, T. Limpaiboon, S. Proungvitaya |
| PS-08 | *In vitro* anti-tumor promoting effect of annonacin from *Annona muricata* on Raji cell<br>M.R. Md Roduan, R. Abdul Hamid, N. Mohtarrudin, C.Y. Kqueen |
| PS-09 | Downregulation of EMMPRIN by specific intrabody induces apoptotic cell death in human colorectal cancer cell line Caco-2<br>P. Thammasit, S. Sangboonruang, W. Kasinrerk, C. Tayapiwatana, K. Tragoolpua |
| PS-10 | The effect of *Cratoxylum formosum* subsp. pruniflorum (Kurz.) Gogel extracts on oral cancer cell lines<br>B. Promraksa, J. Daduang, P. Chaiyarit, T. Khampitak, P. Boonsiri |

| *Abstract No.* | *Title of Presentation* |
|---|---|
| PS-11 | Possible antidiabetic mechanism of action of *Phaleria macrocarpa* methanol-chloroform extract in HepG2 cells<br>N.A. Abdullah, M.A Dollah, A. Akim, S. Sukardi |
| PS-12 | The association between *GPX3* gene polymorphism (rs1946234) and diabetes mellitus in Thais<br>N. Decharatchakul, C. Settasatian, N. Settasatian, P. Yongsakulchai, I. Sarutipaiboon, R. Rattanatham, P. Pitivejthurakit, S. Khaichaiyaphum, P. Boonsiri |
| PS-13 | Monocyte-derived microparticles with severity of vascular stenosis and risk factors for coronary artery disease<br>S. Howhan, N. Komanasin, A. Jumnainsong, N. Settasatian, C. Settasatian, U. Kukongwiriyapan, P. Intharapetch, V. Senthong |
| PS-14 | Hemoglobin E in Southeast Asian populations: Haplotype and phylogenetic analyses<br>W. Jomoui, G. Fucharoen, K. Sanchaisuriya, S. Fucharoen |
| PS-15 | Abnormal hemoglobins in northern Thailand: Molecular and hematological characterization<br>S. Panyasai, G. Fucharoen, S. Fucharoen |
| PS-16 | Molecular and hematological features of adolescent and adult thalassemia patients in Northeast Thailand<br>P. Prayalaw, N. Teawtrakul, A. Jetsrisuparb, S. Pongudom, G. Fucharoen, S. Fucharoen |
| PS-17 | Mitochondrial ferritin expression in erythroid cells of nonsplenectomized and splenectomized patients with beta-thalassemia/HbE<br>Y. Suebpeng, A. Jetsrisuparb, S. Fucharoen, A. Tripatara |
| PS-18 | KLF1 mutations and variability of hemoglobin F expression in homozygous Hb E<br>W. Tepakhan, S. Yamsri, G. Fucharoen, K. Sanchaisuriya, S. Fucharoen |
| PS-19 | Characterization and functional studies of a leukocyte surface molecule recognized by monoclonal antibody FE-1H10<br>S. Khummuang, S. Pata, W. Kasinrerk |
| PS-20 | Screening of immune cells toxicity of ethanolic extract from Tetracera loureiri (Fin & Gagnep.) Pierre ex Craib<br>M. Khamphio, N. Weerapreeyakul, S. Barusrux |
| PS-21 | Establishment of genetically modified induce pluripotent stem cells for HIV-1 gene therapy<br>S. Saoin, W. Khamaikawin, S. Nangola, S. Sakkhachornphop, R. Rungsiwiwut, N. Israsena, C. Tayapiwatana |

| *Abstract No.* | *Title of Presentation* |
|---|---|
| PS-22 | Smoking, *MT2A* polymorphisms, and expression of *MT2A* in peripheral blood leukocyte<br>I. Sarutipaiboon, C. Settasatian, N. Settasatian, P. Yongsakulchai, R. Rattanatham, N. Decharatchakul, S. Khaichaiyaphum |
| PS-23 | Increased frequency of KIR genes in group B haplotype in HIV-1 infected patients lacking immunological recovery regardless of viral suppression<br>Y. Wutti-in, H. Thananchai, N. Leetrakool, W. Kunacheewa, P. Vongchan |
| PS-24 | Discovery of a novel source of chloramphenicol production by endophytic S*treptomyces* SUK 25 sp., isolated from Z*ingiber spectabile a*gainst Methicillin Resistance *Staphylococcus aureus* (MRSA)<br>M.M. Alshaibani, J. Jalil, N.M. Sidik, N.M. Zin |
| PS-25 | Comparative analysis of physical characteristics of *Escherichia coli* isolated from urine of patients with kidney stone disease and urinary tract infection<br>P. Amimanan, R. Tavichakorntrakool, P. Sribenjalux, A. Lulitanond, V. Prasongwattana, C. Wongkham, V. Thongboonkerd, P. Boonsiri, S. Sungkeeree |
| PS-26 | Evaluation of *Piper Aduncum* linnaeus based repellent formulations against Dengue vector in laboratory<br>S.N.H. Mamood, H. Othman, S.B. Budin, A.R. Ghazali, M.H. Zulfakar |
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## PS-01

### Carcinogenic liver fluke secretes exosomes that promote cholangiocytes to adopt a tumorigenic phenotype

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Throughout East Asia there is a strikingly high prevalence of cholangiocarcinoma (CCA - hepatic cancer of the bile duct epithelia) in regions where the human liver fluke, *Opisthorchis viverrini*, is endemic. Indeed, no stronger link between a human malignancy and a parasitic infection occurs than that between CCA and *O. viverrini* infection. A combination of processes are thought to drive liver fluke infection towards cancer, including chronic biliary inflammation caused by resident flukes and active release of excretory/secretory (ES) proteins by the parasite and their subsequent entry into and potential transformation of biliary epithelial cells. Until now, the molecular mechanisms by which these processes occur were mostly unknown. Here we show that adult *O. viverrini* secrete microvesicles that possess the hallmark features of exosomes, notably their shape, size and protein content, including the presence of tetraspanin transmembrane proteins. Using ultra-resolution fluorescence microscopy we showed that *O. viverrini* secreted exosomes were internalized by a human cholangiocyte cell line. Internalization of exosomes resulted in cholangiocyte proliferation and secretion of IL-6, a hallmark cytokine of chronic liver fluke infection, periductal fibrosis and CCA. Exosome uptake by cholangiocytes induced major changes in expression of proteins associated with processes such as phagocytosis, would healing and cancer. Finally, we showed that antibodies to the extracellular loop 2 region of a recombinant *O. viverrini* surface tetraspanin completely blocked the uptake of *O. viverrini* exosomes by cholangiocytes, highlighting a novel potential approach to vaccine development for this chronic infectious cancer. These findings are the first to implicate exosomes from a multicellular pathogen in the disease process, and reveal novel molecular mechanisms of immunopathogenesis and tumorigenesis.

## PS-02

### Anti-senescence activity of a cancer-causing protein cyclin D1

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Cyclin D1 is an oncoprotein (cancer-causing protein) that has many functions regarding cancer cell existence, such as promoting cell division, DNA repairing. Thus, it is possible that cyclin D1 may have other functions in promoting cancer. Currently, there are many studies of cyclin D1 because cyclin D1 could be a main target for anticancer therapy in the future. Cyclin D1 overexpresses in many human cancers. It was found that cell with shRNA specific to cyclin D1 makes cell stop growing and changes morphology similar to senescence cell. The major cause of the cells into senescence state is accumulation of excessive amounts of Reactive Oxygen Species (ROS). This research aims to study the difference of a number of reactive oxygen species between sh-D1 (shRNA specific to cyclin D1) and control (wild type or non-target: shRNA specific to GFP). It was tested with breast cancer cells (MCF-7) and cervical cancer cells (HeLa). Measurement of ROS used both flow cytometry and fluorescence spectrophotometer to obtain accurate results and more reliable to confirm each other. The results showed that amount of ROS had increased in Sh-D1 more than control. Cyclin D1 may play a role in reducing amount of ROS and leads to anti-senescence activity of MCF-7 cells and HeLa.

## PS-03

### LAMP-gold-nanoparticle technique as a simple and rapid detection method of HPV16

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Human papilloma virus (HPV) type 16 is an important cause of cervical cancer found in Thai women and worldwide. Polymerase chain reaction method is widely used for detection DNA of HPV but time-consuming and complicated instrument requirement are its limitations. Loop-mediated isothermal amplification (LAMP) is an alternative method for HPV detection under isothermal conditions which give turbid-amplified product. However, the turbidity detection is not easy to observe by naked eye. Thus, the study is aimed to develop an easy detection method of HPV type 16 by using LAMP-AuNP techniques which red color gold-nanoparticle (AuNP) attached with DNA probe (DNA-labelled AuNP) was used to develop color changing from red to blue for positive result. Tissue specimens (n=74), collected from Srinagarind hospital during the year 2006-2007, were tested for HPV 16 genotype by nested PCR (positive HPV=44, negative HPV=30). The optimized condition of this new LAMP-AuNP technique was 40 min at 65°C. After LAMP amplification completed, its products were hybridized with AuNP, and then detected by addition of magnesium salt. Positive result is based on prevention of a magnesium sulfate salt induced DNA-labelled AuNP color change from red to blue due to hybridization with complementary DNA amplicons that arise from LAMP reaction. The results showed that the sensitivity of LAMP-AuNP techniques was 100 copies of HPV 16 plasmid DNA and no cross-reaction with the other HPV type was observed. All the positive HPV 16 specimens (n=44) gave positive results by LAMP-AuNP techniques. The sensitivity, specificity, positive predictive value and negative predictive value of the LAMP-AuNP technique were all 100% whereas sensitivity of LAMP technique, by turbidity observation, was 84.09%. Therefore, LAMP-AuNP technique is better than LAMP technique. In conclusion, the LAMP-AuNP technique is a simple, rapid, high sensitivity, and it may be useful for detection of HPV16 in resource-limited hospitals or field study.

## PS-04

### Anti-apoptotic parasite's molecule, *opisthorchis viverrini*-thioredoxin-1 related human apoptosis signal regulating kinase-1 in cholangiocytes cell

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*Opisthorchis viverrini* is a carcinogenic human liver fluke endemic in Thailand, Lao PDR, Cambodia and Vietnam. Infection is acquired by eating uncooked freshwater cyprinoid fish and can induce cholangiocarcinoma. This study investigated anti-apoptotic effects of liver fluke thioredoxin-1 (Ov-Trx-1) including interaction with human ASK-1 in cholangiocytes under oxidative stress. The Ov-Trx-1 may play a role in anti-apoptosis involve human ASK-1.

Human cholangiocyte line, H-69, were incubated with recombinant Ov-Trx-1 for 24, 48 and 72 h, after which $H_2O_2$ was added to 300 μM. H69 cells were measured for apoptosis by flow cytometry (annexin-V alexa488/PI), Human Apoptosis Real-time PCR array, Real-time PCR for Specific genes in MAP3K/ASK-1 signaling pathway and measuring growth index by xCELLigence, Immunoprecipitation and live imaging by confocal microscopy.

Ov-Trx-1 showed anti-apoptotic activity in H69 cells induced by $H_2O_2$ by Ov-Trx-1 incubated cells showed decreasing mRNA expression of apoptosis and increasing anti-apoptosis-related genes including induction and positive regulator of apoptosis genes (FASLG, GADD45A, TNFSF8, BAD, DIABLO, TP53BP2, BCL10, FAS, TNFRSF10A), regulation genes (AKT1, NOD1, TRAF2, TRAF3), the death domain and DNA damage genes (FADD, TNFRSF10B, CIDEA, TP73). The multiple groups showed gene under expression in MAP3K/ ASK-1/MAP2K/3, 4, 6, 7/MAPK/ JNK 1, 2/ P38K-α, δ cascades. Immunolocalization of the Ov-Trx-1 in the cells was done. Immunoprecipitation of cell lysates was analyzed by immunoblotting with specific primary antibodies (anti-human ASK-1, anti-His, anti-Ov-Trx-1) was showed Ov-Trx-1 bind human ASK-1. Then measured cell index by xCELLigence was showed Ov-Trx-1 can help cell survived when adding peroxide.

In conclusion, Ov-Trx-1 may play important role in anti-apoptosis via ASK-1 in liver fluke infection related cholangiocarcinogenesis in oxidative stress condition.

## PS-05

### Physical mapping between Y-box DNA/RNA-binding factor, YB-1 truncated proteins and multivalent 11 Zinc Fingers Transcriptional Factors, CTCF and BORIS in Multiforme RGBM cell lines

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CTCF is a unique, highly conserved and ubiquitously expressed 11 zinc finger (ZF) transcriptional factor with multiple target site. It is able to bind to various target sequences to perform different regulatory roles including promoter activation or repression, creating hormone-responsive gene silencing element, and functional block of enhancer-promoter interactions. The binding of CTCF to the essential binding site is through the combination of different ZF domain. On the other hand, BORIS for Brother of the Regulator of Imprinted Sites, which expressed only in the testis and certain cancer cell line is homology to CTCF 11 ZF domains. Since both transcriptional factors share the same ZF domains hence there is a possibility for both to bind to the same target sequences. In this study, both in-vivo and in-vitro interaction of these two proteins to multifunctional Y-box DNA/RNA-binding factor, YB-1 was determined. The protein-protein interaction between CTCF/YB-1 and BORIS/YB-1 were discovered by Co-immunoprecipitation (CO-IP) and pull-down assays through reciprocal experiment from RGBM total cell lysate. The **in vivo** interaction assay showed that CTCF results showed that both CTCF and BORIS were able to interact with YB-1 in Glioma RGBM cell line. In vitro interaction assay showed that the interaction CTCF TO YB-1 occurred through ZF domains and CSD. To the best of our knowledge, this is the first findings demonstrating the ability of BORIS and YB-1 to form a complex *in vivo*.

## PS-06

### Uptake rate of colorectal cancer screening in Thailand and effects of user fee

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In Thailand, colorectal cancer (CRC) is a common cancer with a rapid increase in the incidence rate observed over past twenty years. A nationwide CRC screening program is under consideration. The proposed program employs a fecal immunochemical test (FIT) as a first-line screening tool; those with a positive test will be referred to have colonoscopy. Colonoscopy can also be used as a screening test per se. Although evidence shows that both FIT and colonoscopy are useful, the effectiveness of overall screening program depends heavily on an actual uptake rate. Hence, estimates are required for the uptake rate in order to plan for funding and program delivery. Using willingness to pay (WTP) technique, this study aims to estimate uptake rates of each screening test with and without out-of-pocket user fee and its price elasticity of demand.

Interviews were arranged with 437 individuals aged 50-69 attending the primary care clinic of Songklanagarind Hospital during June-August 2013. Subjects were provided with evidence-based information on the screening tests and asked if they would be willing to participate in screening program with each test. Among those willing to participate, double-bounded WTP questions were asked followed by an open-ended question to elicit WTP for both tests.

Without out-of-pocket user fee, the estimated uptake rates for FIT and colonoscopy were 74.2% and 55.6%. Fifty-nine percent and 46.5% of subjects were willing to participate in paid screening programs with FIT and colonoscopy respectively. Among those willing to pay for screening, the median WTP were 300 baht (≈ 9.4 USD) for FIT and 3,000 baht (≈ 93.8 USD) for colonoscopy. Price elasticity of demand for FIT at user fee equal to the median WTP was -1.16 and that for colonoscopy was -1.40. In other words, both screening tests were price elastic—i.e., 1% decrease in user fee leading to more than 1% increase in demand for screening. However, up to the certain user fees—58.7 baht (≈ 1.8 USD) for FIT and 471.3 baht (≈ 14.7 USD) for colonoscopy—further decrease in user fee was unable to increase the demand for screening among those willing to pay.

For the mass screening in Thailand both with and without service fee, FIT would therefore give better uptake. If fee is to be introduced, uptake rate will increase with decreasing fee but the pricing policy can only partially solve the problem of limited uptake.

## PS-07

### Total serum bile acids as a potential marker for the diagnosis of cholangiocarcinoma

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Diagnosis of cholangiocarcinoma (CCA) is difficult in patients without jaundice (low total serum bilirubin). The aim of this study is to examine the feasibility of using total serum bile acids (TSBA) as an aid in the diagnosis of CCA.

Sixty cases of CCA with total serum bilirubin ≤ 2 mg/dL (called low total bilirubin; LTB), 35 cases of CCA with total serum bilirubin > 2 mg/dL (called high total bilirubin; HTB) and 115 healthy controls were measured for TSBA by Beckman Synchron CX4 clinical chemistry analyzer. Liver function tests such as serum cholesterol, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were also examined.

The results showed that the TSBA of CCA patients with LTB and HTB were both higher than in healthy controls. We also found a significant correlation between TSBA and bilirubin in CCA patients with HTB. Interestingly, no correlation between TSBA and bulirubin was found in CCA patients with LTB. The cut-off value of TSBA determined by the receiver operating characteristic curve analysis of CCA patients with LTB was >6.05 μmol/L. The sensitivity and specificity were 46.7% and 84.4%, respectively. In addition, the ALP levels were significant difference between high TSBA and low TSBA groups. Moreover, the combination of high TSBA and high ALP levels showed increased specificity to 97.4%.

In conclusion, TSBA might be useful for the diagnosis of CCA patients without jaundice.

## PS-08

### *In vitro* anti-tumor promoting effect of annonacin from *Annona muricata* on Raji cells

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Significant increment of global cancer rates through the years, has prompted the chemoprevention based on natural products as an alternative in the development of anticancer drugs. One of the renowned Malaysia's herbs called *Annona muricata* has been widely used traditionally against various condition and illnesses. Several *Annona* species had exhibited promising cytotoxicity against various cancer cell lines. One of the Annonaceous acetogenins called annonacin is the promising antitumor that is found only in the plant family of Annonaceae. This study was aimed at determining the effect of annonacin against tumor promotion, *in vitro*. Annonacin was subjected to inhibition of *Epstein-Barr* virus early antigen (EBV-EA) activation assay induced by the tumor promoter 12-O-tertadecanoylphorbol 13-acetate (TPA). In prior, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to determine the $IC_{50}$ of annonacin against Raji cancer cells. $IC_{50}$ of annonacin was found to be less than 2 µM, indicating its higher toxicity towards the cancer cells. Furthermore, annonacin also showed a moderately active inhibitory effect on the EBV-EA induction using Raji cells at 1000 and 500 molar ratio to TPA. The present findings reported cytotoxicity and antitumor promoting effect of Annonacin, which is a major acetogenin found in *Annona muricata*. Further investigations on in vivo studies are needed to confirm the properties.

## PS-09

### Downregulation of EMMPRIN by specific intrabody induces apoptotic cell death in human colorectal cancer cell line Caco-2

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Intrabodies, the advances in the field of antibody engineering, are being developed and represented a potentially attractive clinical approach for cancer therapy. Extracellular matrix metalloproteinase inducer (EMMPRIN), also known as CD147, is a widely expressed multifunctional transmembrane glycoprotein. Overexpression of EMMPRIN has been implicated in many cancers and offers a specific target for cancer therapy. In this study, we present the downregulation of EMMPRIN cell surface expression by scFv-M6-1B9 intrabody and thereby affect the programmed cell death response in colorectal cancer cells, Caco-2. Elevation of apoptotic bodies in sub-G1 peak, phosphatidylserine externalization and DNA fragmentation were determined in Caco-2 cells expressing scFv-M6-1B9 intrabody. The intrabody transduced Caco-2 cells showed a marked induction of intrinsic apoptosis pathway through a reduction of Bcl-2, leading to the translocation of cytochrome c from mitochondria to the cytosol and also the dramatic activation of caspase-3 by real-time RT-PCR and western blotting analysis. Our results indicate that downregulation of EMMPRIN by scFv-M6-1B9 intrabody may establish as a promising tool in the new therapeutic strategy approach in colorectal cancer treatment.

## PS-10

### The effect of *Cratoxylum formosum* subsp. pruniflorum (Kurz.) Gogel extracts on oral cancer cell lines

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Oral cancer is one type of head and neck cancer which 90% are squamous cell carcinoma. Chemotherapeutic drugs, such as cetuximab and 5-FU, in combination with surgery cause drug resistance and side effects. Alternative medicine may be a method of choice to reduce drug side effect. *Cratoxylum formosum* subsp. pruniflorum (Kurz.) Gogel (Teawdang) is normally consumed in Northeast of Thailand. It contains phytochemicals especially phenolic compounds which also play important role in human health effects including antioxidant, anticancer, etc. Therefore, we aim to determine the cytotoxicity of *Cratoxylum formosum* subsp. pruniflorum (Kurz.) Gogel on oral cancer cell lines. In this study, Teawdang, purchased from Khon Kaen market during June-October 2013, was extracted in hexane (CH), ethyl acetate (CE) and methanol (CM). The studied oral cavity cell lines were ORL-48 and ORL-136 which have MDM2 and EGFR overexpression, respectively. Among all extracts, CM crude extract had the highest phenolic content (161.63±12.85 µg gallic acid equivalent/g dry weight) and flavonoid content (495.00±55.49 µg quercetin eqivalent/g dry weight. Inhibitory concentration (IC50) of CM on ORL-48 cell line (290.1 µg dry wt/mL) was higher than the others. For ORL-136 cell line, CE extract exhibited the highest IC50 (296.00 µg dry wt/mL) compare to the other extracts. The effect of CM and CE on these cell lines may be from different phytochemical content. Investigation of bioactive compounds in these extracts should be further studied.

## PS-11

### Possible antidiabetic mechanism of action of *Phaleria macrocarpa* methanol-chloroform extract in HepG2 cells

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Recently, much attention has been paid to the discovery of natural products that may be advantageous in reducing the risks of diabetes. Diabetes is known can be controlled using natural products. *Phaleria macrocarpa* has been used traditionally to treat diabetic mellitus, however the mechanism of action of which *Phaleria macrocarpa* reduces the blood glucose still remains to be elucidated. Hence, the present study focuses on finding the possible mechanism of actions of *Phaleria macrocarpa* extract on glucose uptake and its possible mechanism of action. HepG2 cells were cultured in RPMI and in the presence of *Phaleria macrocarpa* fruit extracts (0, 0.01, 0.1 and 1 mg/mL) or metformin (1 mg/mL) for 24 hours. Insulin (100nM) was used as positive control. Glucose uptake assay was evaluated in the presence of 2-Deoxy D- [1-2, $3^{H}$ (N)] glucose (0.1μCi/well) for 15 minutes. Wortmannin and genistein were used as inhibitors to study the possible mechanism of *Phaleria macrocarpa* action. Glycogen synthesis was assayed by adding [$^{14}$C]-glucose (0.1μCi/well) to the extracellular medium followed by isolation of cellular glycogen. Glycogen synthase activity was determined by measuring the incorporation of [$^{3}$H]-UDP-glucose (0.1μCi/well) into purified glycogen. In the present study, *Phaleria macrocarpa* methanol-chloroform extract demonstrate the ability to enhance glucose uptake activity up to five-fold increment. All treatment doses (0.01, 0.1 and 1 mg/mL) showed significantly increased in glycogen synthesis and glycogen synthase activity compare to negative control group ($p<0.05$). In inhibitors study, all treatment doses significantly reduced the glucose uptake activity ($p<0.05$) suggesting the possible involvement of PI3-kinase pathway and protein tyrosine kinase pathway which seen in insulin action pathway. This study suggests that *Phaleria macrocarpa* extract its action possibly acting on the same mechanism as of insulin.

# PS-12

## The association between *GPX3* gene polymorphism (rs1946234) and diabetes mellitus in Thais

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**Introduction:** Diabetes mellitus (DM) is a major risk factor for cardiovascular diseases (CVD). It is characterized by chronic hyperglycemia resulting from defects in insulin action (insulin resistance) and insulin secretion. The prevalence of DM in Thai population is 280 cases per 100,000 individuals. Oxidative stress plays major role in the pathogenesis of DM by induction of β-cell dysfunction and insulin resistance. Glutathione peroxidase-3 (GPX3) is one of the prominent antioxidant enzymes involving in the detoxification of hydrogen peroxide and lipid hydro-peroxides, the causes of insulin resistance. The *GPX3* mRNA is found in kidney, lung heart, liver, and adipose tissue. Genetic polymorphism within *GPX3* promoter region (rs1946234; *-1006A>C*) has been reported to alter the expression of *GPX3* that may implicate in insulin resistance. However, the association of *GPX3* gene polymorphism with DM has remained undetermined. The aim of this study was therefore to investigate the association between *GPX3* gene polymorphism (rs1946234) and DM in Thais.

**Method:** A total of 397 community-based Thai subjects (average age: 51.6±10.1 years; 242 males and 155 females) were recruited, divided into 376 non-DM and 21 DM individuals, and determined for biological parameters. *GPX3* gene polymorphism (rs1946234) was determined in genomic DNA using allele specific polymerase chain reaction (AS-PCR) technique.

**Result:** The respective frequencies of *A* and *C* alleles in studied subjects were 85.0% and 15.0%. As compared with *AA* genotype, *C* allele carriers had significantly higher fasting blood sugar (FBS) level (p=0.035), but presented with significantly lower low-density lipoprotein cholesterol (LDL-C) level (p=0.034). There were no differences for other lipid parameters, serum high-sensitive C-reactive protein (hs-CRP), and malondialdehyde (MDA) levels among different genotype groups. By comparing between non-DM and DM individuals, the *C* allele carriers were found to associate with the increase risk of DM with OR = 3.08 (95% CI 1.27-7.47). However, despite showing association with LDL-C level, this polymorphism presented no association with dyslipidemia.

**Conclusion:** This study demonstrated the association of *GPX3* genetic variation, rs1946234, with the increase risk of DM. This might result from the lower level of *GPX3* expression and oxidative protection in *C* carriers.

# PS-13

## Monocyte-derived microparticles with severity of vascular stenosis and risk factors for coronary artery disease

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**Introduction:** Monocytes play an important role in the atherosclerosis, leading to the processes that mediate the atherosclerotic plaque progression and vascular stenosis. During atherosclerotic process, the activation of monocytes occurs and leads to monocyte-derived microparticles (MMPs) production. This study therefore aimed to investigate the relationships among levels of MMPs, degree of coronary stenosis and cardiovascular risk factors.

**Methods:** Forty-seven subjects with age ≥35 years and clinically suspected for CAD underwent coronary angiography at the Cardiac Catheterization Unit, Queen Sirikit Heart Center of the Northeast Hospital, Khon Kaen University were recruited in the study. Based on Gensini score, the patients were classified into individuals with severe stenosis (Gensini score = 32-168) and control group (Gensini score = 0 or was diagnosed as normal coronary artery). MMPs levels were measured by flow cytometry using as the marker.

**Results:** MMPs levels in severe stenosis were significantly higher than those in the control group (11.8±7.6 vs 23.1±13.1 particles/µL respectively, $p$=<0.001). Significant increase in Gensini scores were demonstrated in subjects with hypertension, dyslipidemia and smoking ($p$=0.002, 0.002 and 0.033, respectively). The MMPs levels were also significantly elevated in the subjects with those risk factors compared to individuals without risk factors (22.5±13.0 vs 11.4±6.2 particles/µL, $p$=0.004, 20.7±12.5 vs 8.6±3.7 particles/µL, $p$=0.025 and 24.2±16.7 vs 16.8±9.4 particles/µL, $p$=0.043)

**Conclusions:** Increase in MMPs levels may be related to the atherosclerotic plaque progression and was associated with the risk factors for cardiovascular disease.

## PS-14

### Hemoglobin E in Southeast Asian populations: haplotype and phylogenetic analyses

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Hemoglobin (Hb) E is the most common Hb variant in many countries of Southeast Asia. We reported β-globin gene haplotypes associated with Hb E gene in Cambodian, Laos and northeast Thai populations and constructed a phylogenetic tree based on these haplotypes. A total of 382 chromosomes in Thai, 84 chromosomes in Cambodian and 26 chromosomes in Laos were investigated. Seven DNA polymorphisms within β-globin gene cluster were examined using PCR-RFLP assay. Three most common haplotypes including haplotype 1; (- + - + + + -) haplotype 2 (+ - - - - + -) and haplotype 3 (- + - + + - +) were found at 62.6%, 20.3% and 11.6%, respectively. Other haplotypes were also encountered with rarer frequencies. Phylogenetic tree constructed using the DendroUPGMA method indicated that Hb E on haplotypes 1 and 2 on the framework 2 chromosome detected in Thai, Laos and Cambodian are recent haplotypes and that of haplotype 3 on framework 3 chromosome identified mainly in Cambodian is the older one. These results indicated that there are at least two origins of Hb E with different evolutionary events among Southeast Asian populations which could be responsible for a high prevalence of this Hb variant in the region.

## PS-15

### Abnormal hemoglobins in northern Thailand: Molecular and hematological characterization

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To provide epidemiological data on abnormal hemoglobin (Hb) in the upper northern Thailand, we have conducted study on abnormal Hbs found in 8 provinces during June 2012 – January 2014. Hb patterns were examined using Hb-HPLC and Capillary electrophoresis. The corresponding mutations on α-, β- and δ-globin genes were identified using allele specific PCR and DNA sequencing. Two hundred and eleven samples with abnormal HPLC chromatograms or capillary electrophoregrams were recruited. Hb and DNA analyses identified altogether 14 different abnormal Hbs. Five α-chain variants including Hb Q-Thailand, Hb Hekinan, Hb Siam, Hb Beijing and Hb Kawachi were detected in 40 (19.0%), 8 (3.8%), 2 (0.9%), 1 (0.5%) and 1 (0.5%) cases, respectively. Seven β-chain variants including Hb Hope, Hb Tak, Hb J-Bangkok, Hb S, Hb G-Makassar, Hb Korle-Bu and Hb C were observed in 115 (54.5%), 30 (14.2%), 3 (1.4%), 3 (1.4%), 1 (0.5%), 1 (0.5%) and 1 (0.5%) cases, respectively. Two δ-chain variants were identified in 5 subjects comprising of 4 (1.9%) subjects Hb $A_2$-Melbourne ($\delta^{43\ \underline{G}AG->\underline{A}AG}$) and a novel δ-chain variant, namely the Hb $A_2$-Lampang ($\delta^{47\ \underline{G}AT->\underline{A}AT}$) was identified the remaining subject. Several genetic interactions between these Hb variants with thalassemias were encountered and associated hematological phenotypes were recorded. These findings indicate genetic heterogeneity of abnormal Hb among the upper northern Thai population which is difference from those reported in other parts of the country. Basic information obtained in this study should prove useful in laboratory diagnostics and genetic counseling as well as further population genetic study of hemoglobinopathies in the region.

## PS-16

### Molecular and hematological features of adolescent and adult thalassemia patients in Northeast Thailand

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We have studied the molecular basis and hematological features associated with thalassemia intermedia found in adolescent and adult patients in Northeast Thailand. A total of 309 subjects including 175 with Hb E-β-thalassemia, 1 with homozygous $\beta^0$-thalassemia and 133 with Hb H disease and Hb H disease with Hb E. For β-thalassemia, eleven mutations including codons 41/42 (-TTCT), codon 17(A→T), codons 71/72(+A), IVS I#1 (G→T), IVS I#5 (G→C), IVS II#654 (C→T), -28 (A→G), codon 26(G→T), codon 35(A→G), codons 33/34 (-G) and -90 (C→T) were detected. Co-inheritance of the -158 (C-T) $^{G}\gamma$-*Xmn* I polymorphism was found to be associated with increased Hb F expression and milder hematological severity. For α-thalassemia, 17 patients were found to be deletional and 50 with non-deletional Hb H disease with Hb Constant Spring or Hb Paksé. Three patients were suffered from the EEBart's disease. The remaining 63 subjects were found to be patients with the EABart's disease. Associated hematological parameters of these thalassemia genotypes and genotype-phenotype relationships are discussed. The information on genetic interaction, molecular basis and hematological phenotypes associated with these common genetic disorders should prove useful for prediction of the clinical outcome and improving genetic counseling of the patients as well as facilitating the prevention and control program of thalassemia in the region.

## PS-17

### Mitochondrial ferritin expression in erythroid cells of nonsplenectomized and splenectomized patients with beta-thalassemia/HbE

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**Introduction:** Mitochondrial ferritin is a new form of ferritin located inside the mitochondria. Mitochondrial ferritin gene (*FTMT*) has high similarity to H-ferritin cDNA sequences. The expression of mitochondrial ferritin protein has been reported in reticulocytes of patients with sideroblastic anemia. It is thought that the function of mitochondrial ferritin is to protect the mitochondria from iron generated free reactive oxygen species. The aims of this study were to investigate *FTMT* mRNA levels at steady state in erythroid cells of nonsplenectomized and splenectomized patients with beta-thalassemia/HbE compared with normal controls.

**Methods:** The levels of *FTMT* mRNA from erythroid cells and MDA in the serum from beta-thalassemia/HbE patients with regular transfusions and iron chelation therapy (30 nonsplenectomized and 30 splenectomized) and 30 normal individuals were assessed by reverse transcription real-time PCR and thiobarbituric acid-reactive substances assay, respectively. Ferritin levels in the plasma were determined by enzyme-linked immune absorbent assay, serum iron and unsaturated iron binding capacity by spectrometry and the results of the two tests were used to calculate transferrin saturation.

**Results and Conclusion**: It was found that non-splenectomized and splenectomized patients with beta-thalassemia/HbE had significantly higher levels of *FTMT* mRNA, MDA, plasma ferritin and transferrin saturation than normal controls. In addition, there were significant differences in levels of MDA, plasma ferritin between splenectomized and non-splenectomized patients with thalassemia, but the percentages of transferrin saturation between the two patient groups were not significantly different. Moreover, no correlation was found between relative *FTMT* expression and serum MDA, plasma ferritin, transferrin saturation in beta-thalassemia/HbE patients. The findings suggest that the expression of *FTMT* in these patients is not regulated by iron.

## PS-18

### KLF1 mutations and variability of hemoglobin F expression in homozygous Hb E

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Hemoglobin (Hb) E is the most common Hb variant found in Thailand and the frequency of 40-50% has been noted in northeastern part of the country. Heterozygous Hb E is asymptomatic and has low production of Hb F. Homozygous Hb E is however associated with mild hypochromic microcytic Rbc and variable Hb F expression. We have examined multiple single nucleotide polymorphisms (SNPs) in the KLF1; an erythroid specific transcription factor and determined their associations with Hb F expression in homozygous Hb E. Study was done on 462 homozygous Hb E subjects and 100 normal control subjects. Allele specific PCR assays were developed for identification of three KLF1 mutations including T334R, R238H and -154 (C>T). The results showed that none of these three mutations were observed among 100 normal control subjects. Among 462 subjects with homozygous Hb E, 307 had high Hb F levels (≥ 5%), 155 had lower Hb F levels (< 5%). DNA analysis identified the KLF1 mutations in 18 cases with high Hb F, including the T334R mutation (9/307 = 2.9%), -154 (C>T) mutation (7/307= 2.3%) and R328H mutation (2/307 = 0.7%). Only one subject in the low Hb F group carried the -154 (C>T) mutation. Although not exclusively, these KLF1 mutations might be one of the genetic factors associated with increased Hb F and in combination could explain the variation of Hb F expression in Hb EE disease in Thai population. Other genetic factors such as HBS1L-MYB and BCL11A regulating Hb F expression in this common genetic disorder remains to be elucidated.

# PS-19

## Characterization and functional studies of a leukocyte surface molecule recognized by monoclonal antibody FE-1H10

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Lymphocytes are cellular components of the immune system that coordinate the immune responses. These cells consist of distinct subsets. The molecules expressed on lymphocyte surface are demonstrated to play key roles in all aspects of their functions. Discovery of new lymphocytes surface molecules, therefore, has impact on the understanding of the function of these cells. To identify novel lymphocyte surface molecule, in this study, we have produced a monoclonal antibody to leukocyte surface molecule (mAb) name FE-1H10. By immunofluorescence staining, molecule recognized by FE-1H10 mAb were demonstrated to particularly express on CD3-CD56+ (Natural killer cells), CD3+CD8+ (Cytotoxic T cells), and T cell lines. During T cell activation, mAb FE-1H10 induced apoptosis of T cell lines. FE-1H10 positive and FE-1H10 negative SupT1 cells were established by using immunomagnetic separation. It was found that, upon cultivation, the expression patterns of molecule recognized by FE-1H10 mAb on the established cells were altered. By Western blotting, mAb FE-1H10 reacted to protein bands having pattern that different from the reported proteins and the results by RT-PCR showed the distinct in cytokine gene expression. Currently, more functional involvement of this molecule on the activity killer cells is exploring. The amino acid sequence of the molecule recognized by FE-1H10 mAb is also under investigated by Mass-spectrometry. In conclusion, we demonstrate here a novel leukocyte surface molecule recognized by FE-1H10 mAb may play an important role in the immune system.

## PS-20

**Screening of immune cells toxicity of ethanolic extract from extract *Tetracera loureiri* (Fin & Gagnep.) Pierre ex Craib**

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*Tetracera loureiri* (Fin & Gagnep.) Pierre ex Craib or Ros-Sukon (RS) is a herbal plant that found at Chulabhorn dam, Chaiyaphum province, Thailand. The previous report showed that the ethanolic extracts of *T. loureiri* were demonstrated strong antioxidant and antihepatoprotective activity from chemical toxicants. To screen for the toxicity effect of RS extracts to immune cells. Peripheral blood mononuclear cell (PBMC) was treated with 10, 25, 50, 250, and 500 µg/ml of RS ethanolic extract and compared to untreated group. The percentage of cell viability was measured by neutral red assay. RS ethanolic extract showed non toxic against to PBMC at the concentration less than 250 µg/ml and also increased cell viability as dose-dependent manner compare to untreated group ($p<0.05$). The 50 µg/ml of RS ethanolic extract showed the increasing of 2.17 folds of cell viability compared to untreated group. In contrast, the higher concentration (500 µg/ml) of RS ethanolic extracts showed completely cytotoxicity to PBMC. RS extracts is safe to PBMC at low concentration. However, further study for understanding the cells toxicity mechanism is still interested.

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## PS-21

### Establishment of genetically modified induce pluripotent stem cells for HIV-1 gene therapy

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Induced pluripotent stem cell (iPSC) technology has provided a promising therapy by production of patient-specific pluripotent stem cells from adult individual. By developing iPSCs to treat HIV, there is the potential for generating a continuous supply of therapeutic cells for transplantation into the patients. According to our research, designed zinc finger protein, namely 2LTRZFP, and ankyrin-repeat protein, AnkGag1D4, have recently been generated with the promising function to inhibit HIV viral replication in early and late stages of infection, respectively. In this study, we aim to apply these two anti-HIV proteins as a HIV gene therapy by using iPSC model. The primary fibroblast cells were initially transduced with third generation lentivirus harboring 2LTRZFP-mCherry and AnkGag1D4-EGFP genes. Fluorescence microscopy analysis revealed two anti-HIV proteins totally expressed in fibroblast cells. The transduced cells were further sorted by FACS and used as the starter cells for reprogramming. Consequently, we successfully produced iPSC based on Sendai virus vectors. Moreover, both of therapeutic genes also existed in the iPSC chromosomes analyzed by Alu-Gag qPCR. For further studies, we will use this genetically modified iPSC to differentiate toward hematopoietic progenitors and subsequently generate iPSC-derived mature CD4+ lymphocytes and macrophages in order to offer a lifelong protection from HIV replication. This approach might provide the potential treatment that could reconstitute of a patient immune system and also resist to HIV-1 infection.

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# PS-22

## Smoking, *MT2A* polymorphisms, and expression of *MT2A* in peripheral blood leukocyte

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**Background:** Smoking is the important risk factor for cardiovascular diseases (CVDs) by inducing oxidative stress and chronic inflammation. Metallothionein (MT) is a low molecular weight metal binding protein. MT plays important role on homeostasis of essential metals, anti-oxidative stress by scavenging the free radicals, and detoxifying toxic metals such as $Cd^{2+}$. *MT2A* mutation has been associated with the incidence of CVDs, the causes of which are related to oxidative stress. Variations within *MT2A*, affecting its expression, may modify the smoking-related oxidative stress. The aim of the present study was to investigate the relationships of *MT2A* polymorphisms and smoking status with *MT2A* gene expression in Thais.

**Methods:** Expression of *MT2A* in peripheral blood leukocyte was investigated using reverse transcription polymerase chain reaction (RT-PCR). The *MT2A* single-nucleotide polymorphisms (SNPs) *rs1612016(A/G), rs28366003(A/G),* and *rs10636(C/G)* were genotyped in both smokers and never-smokers using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Malondialdehyde (MDA) was measured by thiobarbituric acid method. The *MT2A* SNPs and MDA were evaluated for their relationship with expression of *MT2A*.

**Results:** Significant difference of *MT2A* expression were found among never-smokers, former-smokers and current-smokers (p=0.001), with the highest levels in former-smoker. Furthermore, the level of MT2A expression was also associated with *MT2A* gene polymorphism, *rs10636(C/G)*; the expression was higher in homozygous *CC* than *CG* and *GG*, p=0.002. The level of *MT2A* expression was also investigated the relationship with oxidative marker, MDA, and modest correlation was observed.

**Conclusion:** Expression of *MT2A* was associated with both smoking status and polymorphisms of *MT2A*, suggesting the expression was under both environmental and genetic factors.

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## PS-23

### Increased frequency of KIR genes in group B haplotype in HIV-1 infected patients lacking immunological recovery regardless of viral suppression

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During the course of antiretroviral therapy, some HIV-1 infected patients did not get an adequate $CD4_+$T cell recovery, although HIV replication has been suppressed. In this study, we investigated the influence of KIR gene in HIV-1 infected patient receiving cART and maintained suppression of HIV replication without an adequate $CD4_+$T cell recovery (immunological nonresponder : INR) in comparison with those who restored $CD4_+$T cell count more than 200 cells/cu.mm (full responder : FR). The study was focus on Northern Thai population through a case control study. Ninety seven HIV-1 infected patients and 49 healthy individuals of aged-match control group were recruited. *KIR* genotyping was performed by real-time PCR with sequence specific primers. The results showed that the frequency of *KIR3DL1* was significantly higher in FR than INR ($p$=0.006). In addition, frequencies of *KIR2DS2* and *KIR2DS3* were higher in INR than control group ($p$=0.029 and $p$=0.035, respectively). Moreover, INR showed significantly higher frequency of *KIR2DL5* than FR ($p$=0.004) and control ($p$=0.021). Regarding of KIR haplotype, group B haplotypes were more frequent in HIV-1 infected patients. These data suggested that the presence of *KIR3DL1* in FR seem to be potential factor of immune recovery. However, the presence of group B haplotype genes (*KIR2DS2*, *KIR2DS3* and *KIR2DL5*) may be a factor of susceptibility to HIV-1 infection and a risk of poor immunological recovery.

## PS-24

**Discovery of a novel source of chloramphenicol production by endophytic *Streptomyces* SUK 25 sp., isolated from *Zingiber spectabile* against methicillin resistance *Staphylococcus aureus* (MRSA)**

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Increasing antibiotic resistance by pathogenic bacteria is cause for concern, as the rate of discovery for new antibiotics has been in degeneration. The demand for new antibiotics continues to grow due to the rapid spread of antibiotic-resistant pathogens causing life-threatening infections. Methicillin resistance *Staphylococcus aureus* (MRSA) bacterium has ability to gain and express a widespread of virulence factors and antimicrobial resistance determinants that lead to cause a diseases, increase mortality, morbidity and health care costs. The treatment of bacterial infections is increasingly complicated. *Streptomyces* produced over two thirds of the clinically useful antibiotics of natural origin source. Chloramphenicol (Cm) was isolated first time from *Streptomyces venezuelae*. Cm is a potent inhibitor of protein synthesis, which is extremely active against a variety of organisms including Gram positive and Gram negative bacteria. This study aimed to isolate secondary metabolites from endophytic *Streptomyces* SUK 25 sp., which isolated from roots of *Zingiber spectabile*. Molecular identification results using 16S rRNA gene sequencing showed that SUK 25 has 95 % sequence similarity to *Streptomyces omiyaensis* NBRC 13449T which was produced chloramphenicol. Production of secondary metabolites by this strain was optimized through Thronton's media at 7 days incubation with pH 7 and aerated at 140rpm. Isolation, purification and identification was carried out using analytical and preparative HPLC, LC- MS, FT- IR and chemical structure elucidation was carried out by 1-D and 2-D NMR. As a result of this study, SUK 25 appear activity against MRSA ATCC 43300, MRSA ATCC 33591 and MRSA ATCC 49476 during primary and secondary screening. Its biological activity was tested against MRSA ATCC 43300 which indicated good activity with MIC value at 8µg/ ml. LC- MS results show the molecular weight of Cm using ESI -mass values at m/z: 321 [M+H]. FT-IR and NMR results showed the same peaks of commercial standard chloramphenicol. In conclusion SUK 25 isolated from *Zingiber spectabile* is a novel source to produce chloramphenicol and potentially to be used as anti MRSA.

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## PS-25

### Comparative analysis of physical characteristics of *Escherichia coli* isolated from urine of patients with kidney stone disease and urinary tract infection

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**Introduction:** Urinary tract infection (UTI) is well known to be associated with kidney stone disease (KSD). *E. coli* was the most common causative microorganism in urine of patient with KSD and UTI in northeast of Thailand. Therefore, we evaluated the physical characteristics and biofilm formation of *E. coli* isolated from urine of patients with KSD and UTI.

**Methods:** The five pairs of *E. coli* isolated from urine of patients with KSD (KSD group; n=5) and UTI (UTI group; n=5) were selected from the same of antimicrobial susceptibility patterns and also confirmed the genotype by ERIC- PCR. The size of bacterial colonies and length of bacterial cells were measured. The growth rate of *E. coli* was evaluated by using the mid-log phase of growth curve. The biofilm formation was detected under scanning electron microscope.

**Results:** Five pair of *E. coli* isolates from urine of patients with KSD and UTI gave indistinguishable ERIC- PCR patterns. In the KSD group, the ratio of biofilm formation (20%) was lower than those of the UTI group (60%), whereas the size of bacterial colony (2.50±0.5 vs 2.06±0.15 mm), length of bacterial cells (2.26 ±0.13 vs 2.65 ±0.21 nm) and mid-log phase of growth curve (4.6±0.42 vs 3.8±0.31) were not significantly different.

**Conclusions:** Our study provided the descriptive data of the decreasing in the ratio of biofilm formation and some little changes of the size of bacterial colony, length of bacterial cells and mid-log phase of growth curve of KSD group. These results indicated the phenotypic adaptation of *E. coli* in the different environment of host.

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## PS-26

### Evaluation of *Piper aduncum* linnaeus based repellent formulations against Dengue vector in laboratory

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*Piper aduncum* essential oil exhibit repellency activity and has a potential as an alternative for synthetic repellent such as N, N-Diethyl-meta-toluamide (DEET). However, their efficacy reduces upon application due to its high volatility properties. Studies have shown that formulations can improve efficacy of the essential oil as repellent. Therefore, this study was conducted to evaluate the effectiveness of three different formulations that contained *Piper aduncum* essential oil. The prepared formulation in ointment, cream and gel forms containing 10% *P. aduncum* essential oil was tested in laboratory using *Standards and Industrial Research Institute of Malaysia* (SIRIM) bioassay method. It was found that cream formulation containing 10% *P. aduncum* essential oil can provide 80% repellency up to 210 minutes compared to ointment and gel formulations which provides 80% repellency for 150 and 180 minutes respectively. However, no significant different was found for repellency between ointment, cream and gel formulations containing *P. aduncum* essential oil at p= 0.611. As cream formulation provides higher protection compare to other formulations, it can be further develop and commercialize as an alternative to synthetic repellent.

## PS-27

### Antimicrobial susceptibility patterns and distribution of carbapenemase genes among *Acinetobacter baumanii* isolated from patients at UKM Medical Centre

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*Acinetobacter baumanii* is an opportunistic bacterium causing widespread nosocomial infections and tends to be multi-resistant to majority of antibiotics. Tigecycline is a well-known antibiotic that possess a wide-range of activities and is very active in vitro towards a variety of resistant pathogens, including *A. baumanii.* The aim of this study was to evaluate the antimicrobial susceptibility pattern of imipenem and tigecycline against carbapenem-resistant *A. baumanii* (CRAB) as well as to locate the distribution of carbapenemase genes among these *A. baumanii* strains. The Minimal Inhibitory Concentration (MIC) was determined using the E-test method. The presence of carbapenemase genes was evaluated using a PCR method. The results demonstrated that all *A. baumanni* strains exhibit imipenem MIC values of 32µg/mL which indicates resistance. However, the MIC values obtained for tigecycline were 2-6µg/mL, representative of sensitive and intermediate strains. All strains showed to harbor *oxa-51* gene. The use of imipenem and tigecycline would be of beneficial in patients' treatment if the usage indication respective for each antimicrobials were followed as prescribed so as to reduce further emergence of such resistant strains of CRAB in the future.

## PS-28

### Anti-*Pythium insidiosum* activity of bacterial strains isolated from environmental

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Pythiosis is a life-threatening infectious disease in humans and animals caused by the aquatic oomycete**.** Clinical symptoms of the disease in humans have been described into four forms (i) granulomatous and ulcerative lesions involving the skin and subcutaneous tissue of the limb and face, including the periorbital areas; (ii) opthalamic pythiosis causing corneal ulcer or keratitis; (iii) systemic pythiosis with vascular involvement leading to vasculitis, thrombosis and aneurysm; and (iv) disseminated pythiosis involved in the internal organs such as stomach, brain and heart tissue. The purposes of this study were testing of bacteria with an ability to produce metabolites having anti-*Pythium insidiosum* property. A total of bacterial 7 strains were isolated from water resources in northeastern Thailand including *Pseudomonas stutzeri* ST1302, *Acenitobacter baumannii* ST2512, *Acenitobacter baumannii* ST62302, *Enterobacter cloacae* ST3604, *Enterobacter cloacae* ST2503, *Klebsiella pneumonia* ST2501 and *Aeromonas sobria* ST2206. These organisms were tested with *P. insidiosum* SIMI 6666 that isolated from corneal ulcer of pythiosis patients. The methods were used to establish their inhibitory effects on inhibition zone agar. All of bacterias were crude extracted by culturing with optimum condition (37°C, 200 rpm, 72 hr.), with organic solvent. Evaporation and disc diffusion method test for anti-*P. insidiosum* activities were performed. The result found that Anti-*P. insidiosum* activity was detected in all crude extracted of tested bacteria. The inhibition zone varied from 3-6 mm which *A. sobria* ST2206 and *K. pneumoniae* ST2501 gave maximum diameter. *P. stutzeri* ST1302, *E. cloacae* ST3604, and *E. cloacae* ST2503 gave 5 mm diameter while *A.baumannii* ST62302 gave 4 mm diameter. The minimum inhibition zone were found in *A.baumannii* ST2512. The organic solvent that used for extraction were different in each bacteria. Dichloromethane was used for both strains of *A. baumannii* and *P. stutzeri* ST1302.

Ethylacetate was used for the left strains. The results showed the possibility of using the bacterial metabolites against *P. insidiosum*. The results may contribute to the development of antimicrobial drugs/probiotics against pythiosis. Nevertheless further searching for genes involved in the anti-*Pythium* activity will be done**.**

## PS-29

**Molecular typing of *Mycobacterium tuberculosis* isolates from Srinagarind Hospital: Analysis by mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing**

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**Introduction:** Tuberculosis is one of the important infectious diseases which has been distributed around the world. The epidemiological study of *Mycobacterium tuberculosis* based on molecular methods have been developed and widely used to investigate the genetic diversity, control the disease transmission and evaluate drug resistance.

**Objectives:** To evaluate the genetic diversity of *Mycobacterium tuberculosis* isolates from Srinagarind Hospital using MIRU-VNTR typing

**Methods:** The DNA of 27 *Mycobacterium tuberculosis* isolates were amplified by PCR using 24 loci of MIRU-VNTR primer sets. The size of PCR products were analyzed with agarose gel electrophoresis and calculated to allelic numbers using allele calling tables. MIRU-VNTR profiles were analyzed by cluster analysis using UPGMA in the MIRU-VNTR*plus* software provided by international MIRU-VNTRplus database (http://www.miru-vntrplus.org). The discriminatory power index of each and combined MIRU-VNTR loci were showed through allelic diversity (*h*) using the Hunter–Gaston discriminatory Index (HGDI).

**Results:** Genotyping revealed a total of 24 MIRU-VNTR profiles comprising of 2 lineages: 14 East-African Indian (EAI) isolates (51.85%) with 11 profiles and 3 Beijing isolates (11.11%) with 3 profiles. The other 10 unique profiles (37.04%) were not identical to any profile in MIRU-VNTRplus database. In addition, primers Mtub04 (0.6), MIRU04 (0.855), MIRU16 (0.613), Q2163b (0.693), ETRA (0.673), ETRB (0.777), Mtub39 (0.780) and QUB26 (0.704) gave high HGDI. These 8 MIRU-VNTR loci may be appropriate for epidemiology investigation in this region.

**Conclusion:** In this study, we have found this tool really useful for epidemiological and evolutionary studies of *Mycobacterium tuberculosis* and can be used for further study in a large numbers of infections in this region.

## PS-30

### Genetic diversity of selected commercial freshwater fishes based on phospholipase C zeta (PLCζ) expression and muscle protein profiling

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Egg activation is important to help releases the egg from meiotic arrest and blocks polyspermy. It is linked with an increase in intracellular egg calcium ions ($Ca^{2+}$) in almost all species studied and current studies imply that the mammalian sperm factor involved is a sperm-specific phospholipase C zeta, PLCζ. Here, we first reported the identification of PLCζ in the testis and egg of Lampam Jawa. Our findings provide the evidence that PLCζ is present in the species of male and female Lampam Jawa *(Barbonymus gonionotus).* For this study, six types of commercial freshwater fish were selected i.e. Red Tilapia (*Oreochromis sp. Red Tilapia*), Black Tilapia (*Oreochromis mossambicus*), Catfish or Keli (*Ictalurus punctatus*), Silver Catfish or Patin (*Pangasius pangasius*), Snakehead Fish or Haruan (*Channa striata*) and Silver Barb or Lampam Jawa (*Barbonymus gonionotus*). The objectives of this study were to isolate the mRNA from the gonads of freshwater fishes, to identify and amplify the phospholipase C zeta (PLCζ) gene fragments, to sequence the purified DNA fragments and to compare the PLCζ sequence to other PLCζ sequence available in NCBI database, to characterize muscle protein of selected commercial freshwater fish and lastly, to compare phylogenetic trees of 16S rDNA generated. In addition, protein profiles can be used as indicators of evolutionary relatedness. The differences and similarity aspects of fish muscle protein were measured and the relatedness based on protein profile was compared with the relatedness of fishes obtained from 16S rDNA sequences alignment by using dendrogram. The methods used for the expression of PLCζ were RNA extraction, spectrophotometric quantitation of RNA, Two-Step RT-PCR reaction, agarose gel electrophoresis, gel documentation, gel extraction, sequencing and dendrogram. The methods used for muscle protein profiling were sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), gel viewing, muscle's protein banding profile analysis, genetic diversity and lastly comparison of protein profile and 16S phylogenetic tree generated by using dendrogram. For the study of PLCζ expression, male and female Lampam Jawa showed bands at around 420bp in agarose gel electrophoresis that indicated the presence of PLCζ gene and no significant bands were found in other types of fishes used in this study. For muscle protein profiling, the multiple bands of proteins obtained from SDS – PAGE showed similar protein contents among different fish species used in this study. The dendrogram showed the highest percentage of similarity is between Tilapia Hitam and Tilapia Merah which is 84% followed by Haruan and Patin which exhibited less than 84% similarity. Keli had 67% similarity with Haruan, Patin, Tilapia Merah and Tilapia Hitam while Lampam Jawa showed less than 60% similarity with Keli, Patin, Haruan, Tilapia Merah and Tilapia Hitam.

## PS-31

### Protective effects of palm oil tocotrienol rich fraction in reducing sperm damages induced by fenitrothion

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Supplementation of vitamins antioxidant may have a potential in reducing sperm damage induced by organophosphate. Exposure to organophosphate insecticides such as fenitrothion (FNT) has been reported to affect sperm quality. Therefore, present study is carried out to evaluate the effects of Palm oil Tocotrienol Rich Fraction (TRF) which known as a potent antioxidant in reducing the detrimental effects occurring in spermatozoa of FNT treated rats. Mature male Sprague-Dawley rats were divided into four equal groups: control, TRF (200 mg/kg), FNT (20 mg/kg) and TRF (200 mg/kg) combined with FNT (20 mg/kg) for 28 consecutive days. The sperm characteristics, oxidative stress markers as well as DNA damage were evaluated. Supplementation of TRF attenuated the harmful effects of FNT by significantly increased the sperm counts, motility and viability and also decreased the abnormal sperm morphology. The superoxide dismutase activity and reduced glutathione level were significantly increased whereas the malondialdehyde and protein carbonyl levels were significantly decreased in TRF+FNT group compared to rats receiving FNT alone. TRF significantly decreased the DNA damage of sperm in FNT treated rats. TRF showed the potential in reducing the detrimental effects occurring in spermatozoa of FNT treated rats.

## PS-32

### Best reference genes for RT-qPCR in human glial and neural stem cells

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Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) has emerged as robust assay that provide absolute and relative quantification of mRNA transcription levels. The expression of interest gene is usually normalized to an internal standard known as reference genes. Reference genes are genes that are expressed constantly in all cells to maintain essential cellular functions. Ideally, reference genes should exhibit constant expression level across all tissue types and experimental conditions. Unfortunately, different types of tissue exhibit tissue-specific expression profile of reference genes, there is no universal reference gene which could be used in all studies without prior verification. In this study, we investigated the expression level of six candidate reference genes, glyceraldehydes-3-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyl transferase 1 (HPRT1), 60s ribosomal protein large P1 (RPLP1), TATA box binding protein (TBP), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ) and eukaryotic initiation factor 4A (elF4A) in two types of brain cells: glial cell line (SVG p12) and neural stem cell line (hNSCs). The qPCR data was analyzed and compared using two web-based computational programs: NormFinder and Bestkeeper. Analyses by both programs revealed that the most stably expressed reference gene across SVG P12 and hNSCs was elF4A followed by RPLP1. HPRT and TBP were ranked as the least stably expressed genes by NormFinder and Bestkeeper respectively. The validity and accuracy of RT-qPCR are highly dependent on the selection of most stably expressed reference gene, therefore elF4A and RPLP1 were selected as the most ideal candidate reference genes for gene expression studies across SVG P12 and hNSCs.

## PS-33

### The synergistic effect of silk fibroin-gelatin/chondroitin sulfate/hyaluronate scaffold and dynamic compression on cartilage tissue engineering

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The basic approach to cartilage tissue engineering involves the use of cells, scaffolds, and biochemical factors. As known, the microenvironment which supports adult chondrocytes is constantly subjected to mechanical compression. Thus, many studies have considered culture of chondrocytes in a dynamic condition to stimulate mechanical forces for effective improvement of chondrocyte growth. Our present study aimed to investigate the effect of silk fibroin-gelatin/chondroitin sulfate/hyaluronate (SF-GCH) scaffold and dynamic compression culture system on chondrocyte growth. We fabricated silk fibroin (SF) scaffold and SF-GCH blending scaffold that mimic cartilage microenvironment. Human chondrocytes derived from osteoarthritic joints were seeded in scaffolds, cultured for 1 week, and subjected to compression with 10% dynamic strain at 1 Hz, 1 hour/day for 2 weeks. Cartilage specific and dedifferentiation gene expression were evaluated using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). The biosynthetic activities of chondrocytes cultured in scaffolds were assessed by measuring DNA content, glycosaminoglycan (GAG) content and compressive moduli, as well as immunostaining for collagen type II, collagen type I and aggrecan. When dynamic compression was applied, the chondrocyte-seeded scaffold showed significantly increased aggrecan (ACAN) and Collagen X (COLX) gene expression up to five-fold higher than unloading control. In addition, dynamic compression dramatically improved the chondrocyte biosynthesis cultured in SF and SF-GCH scaffolds evidenced by GAG content, GAG/DNA ratio, and immunostaining of collagen type II and ACAN. However, chondrocytes cultured in SF-GCH scaffold under dynamic culture showed higher GAG content and compressive modulus than those in SF scaffold. In conclusion, SF-CCH scaffold and dynamic compression enable the improvement of chondrocyte biosynthesis indicating the great potential of SF-GCH scaffold for cartilage tissue engineering applications.

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## PS-34

### Birth rates in adolescent pregnant women giving birth in the hospitals of Thailand

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The objective of this study was to determine the rates of births in adolescent pregnant women in different regions of Thailand. Data of all pregnant women aged 10 to 49 years, who were admitted to hospitals between October 2010 and September 2011 were anonymously retrieved from the National Health Security Office database Adolescent birth rate by the regions was analyzed. The overall of birth rate per 1,000 adolescents aged 10-14 years and 15-19 years was 1.4 and 43.8, respectively. Respective birth rates per 1,000 were 1.3 and 29.8 in Bangkok, 1.9 and 49.3 in the central, 1.7 and 40.9 in the northern, 1.1 and 44.4 in the northeastern, and 1.1 and 45.1 in the southern regions. Highest birth rate was found among those 19 years (58.3 per 1,000 population). Further investigations are needed to find the reason for this regional discrepancies before proper intervention could be planned.

## PS-35

### n4Studies -- A mobile application for calculation of sample size and power of a study

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Sample size determination in a health science research should be planned in a protocol phase before collecting data to ensure efficiency of the study. When a study reveal no significant difference in major hypothesis testing, it is also important to check the power of that study. Using statistical software on a personal computer (PC) is more convenient than doing manual calculation using the formula. However, using application (app) on a smart device (phone or tablet) is even more convenient. Thus, the aim of this study to developed an app serving these purposes. At this moment, "**n4Studies**" has been developed and made available for free on the iOS "App Store" and Android "Play Store". The app covers sample size and power of a study for various epidemiological study designs including comparison of two population means and proportions, matched and unmatched case-control studies, randomized controlled trial, cohort study, non-inferiority and superiority trial, equivalence trial and so on. **n4Studies** gives the same or similar results as those software on PC such as STATA, PS, epicalc package on R, G*Power, and OpenEpi. Installation, demonstration and explanation of this app to all interested persons will be performed at the poster session.

## PS-36

### Effects of walking training with and without visuotemporal cues in chronic male patients with spinal cord injury

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The sensorimotor deterioration following **s**pinal cord injury (SCI) likely reduces ability of the patients to effectively control movements, particularly while performing a complex task such as walking. The dramatically decrease of rehabilitation length may further affect ability of the patients to obtain an optimal movement. Therefore, an exploration for an effective strategy helping the patients, who were at a chronic stage of SCI, to improve their ability during a short period of time would have vital contribution to rehabilitation practice. The study investigated effects of overground walking training with and without using the visuotemporal cue on levels of functional ability relating to walking ability in 22 chronic male patients with SCI. The subjects were randomly arranged into an experimental or control group in order to receive walking training with or without visuotemporal cue, respectively (11 subjects/ group), for 30 minutes/day, and for 5 consecutive days. Prior to and after the training periods, subjects were assessed for their functional ability using the 10-meter walk test, 6-minute walk test, timed up and go test and five times sit-to-stand test. After 5-day training period, subjects who were trained using the visuotemporal cue showed clearly improvement in their functional ability ($p<0.05$) whereas those in the control group demonstrated the improvement in 10-meter walk test (maximal walking speed) and timed up and go test ($p< 0.05$). The findings suggest the benefit of the incorporation of visuotemporal cue in rehabilitation practice to promote functional ability of the patients. Nevertheless, to confirm learning effects, a further study with a measurement during a retention period is needed.

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## PS-37

### Comparison of health-related quality of life among Thai population with end stage renal disease who have been treated with peritoneal dialysis, hemodialysis, and kidney transplantation

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**Introduction:** End stage renal disease (ESRD) has been encountered in many countries including Thailand. The treatment of ESRD is concerned as a major public health problem in Thailand. Health-related quality of life (HRQOL) is a multi-dimensional concept that includes domains related to physical, mental, emotional and social functions. It is an important indicator of the functioning, well-being and general health perception of individual's life. HRQOL is also the significant marker of the effective medical treatments.

**Material and methods:** The data were collected by Baseline Characteristics and Short Form health survey (SF-36) from 48 ESRD patients who have been treated with peritoneal dialysis, hemodialysis, and kidney transplantation in Ramathibodi Hospital, Mahidol University during October to November 2013. Cross-sectional study analysis was performed.

**Results:** Three methods of ESRD treatments showed similar baseline characteristics except that the mean age of renal transplant patients (42.9 ± 15.1 years) was significantly lower than peritoneal dialysis (53.5 ± 9.7) and hemodialysis patients (56.9 ± 6.3) ($P$ = 0.002). There were statistically significant differences of HRQOL score between the three groups. Kidney transplantation patients had significantly higher physical function scores (85.6 ± 18.4) than hemodialysis (75.9 ± 25.6) and peritoneal dialysis patients (61.3 ± 26.6) ($P$=0.024). Kidney transplantation patients also had significantly higher scores of vitality aspect (75.9±16.4) than hemodialysis (60.9±19.1) and peritoneal dialysis patients (59.3±20.3) ($P$= 0.029).

**Conclusion:** Kidney transplantation showed the greatest scores of almost all aspects excluding role-emotional function in which peritoneal dialysis patients have the highest scores. In contrast, scores of all of aspects in peritoneal dialysis revealed to be the least.

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