Final Report

Research Project
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Genetics and Molecular Biology of Diseases Relevant to Thais
(พันธุศาสตร์และอณูชีววิทยาของโรคที่สำคัญในคนไทย)

By

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Executive Summary

In the past decade, the availability of a complete human genome sequence in couple with newly developed technologies has revolutionized biomedical research to study human diseases. By using the human genome-sequence information and newly developed technologies, we are able to conduct genomic/genetic analyses and molecular biology studies to understand human disease etiology and pathogenesis at a more rapid pace than that was ever occurred in the past. However, as it has been evident that etiology and pathogenesis of same diseases are dissimilar in different populations, it is not possible to use information obtained from the studies in other populations to explain molecular mechanisms and pathogeneses of diseases in Thai patients. Thus, it is unavoidable to conduct direct investigations into these diseases in Thai patients to gain the correct understanding and to design more proper prevention and intervention. Our group has long-term experiences in conducting genetics and molecular biology studies of diabetes, kidney diseases, and dengue virus infection in Thais. Therefore, we take this advantage to focus our research activities to further investigate into these diseases by applying advanced technologies in genetics/genomics and molecular biology. The research activities of our TRF Research Scholar Group can be classified into five projects as follows:

Project I: Genetics/genomics and molecular biology of diabetes mellitus.
Project II: Genetics/genomics and molecular biology of kidney stone disease.
Project III: Molecular mechanism of human kidney anion exchanger 1 trafficking associated with distal renal tubular acidosis.
Project IV: Molecular pathogenesis of dengue virus infected liver cells.
Project V: Production of human single chain antibody variable fragments and peptide inhibitors specific to dengue virus proteins.

In the past three years, our TRF Senior Research Scholar Group achieved the goals in terms of generating new information and knowledge in the areas of our research interests as evidenced by publications in peer-reviewed international journals and presentations in scientific conferences, training graduate (Ph.D. and M.Sc.) students, and extending research collaborations and networks. The following outputs are generated in the past three years of the award:

1. Twenty-eight publications in peer-reviewed international journals,
2. Two publications in peer-reviewed national journals,
3. Three proceedings in international conference book and national journals,
4. Thirty-two abstracts in the international and national journals,
5. Thirty-six presentations in international conferences,
6. Twenty-four presentations in national conferences,
7. Fifteen academic/research awards,
8. Five Ph.D. graduates and eight M.Sc. graduates,
9. Twelve Ph.D. students and six M.Sc. students (currently studying and conducting research in our research group).

Abstract

Human genomic variations can directly cause human diseases or affect them at various stages including disease susceptibility, pathological pathway, disease progression and severity, respond to treatment and recovery. It has been known that same human disease usually occurs from diverse causes and pathological processes attributable to marked genetic heterogeneity in different populations and individuals. Recently, compelling evidence suggests that rare mutations with severe effect are responsible for a substantial portion of complex human disease, and genetic heterogeneity is important at multiple levels of disease causation. Based on this information, we propose that complex human diseases in Thais are caused by rare mutations with severe effect of the responsible genes that possess genetic heterogeneity. Thus, it is necessary to directly investigate into etiologies and pathogeneses of these diseases in Thai patients to understand their natures and to design appropriate interventions. The availabilities of sequence information of human genome and powerful molecular biology techniques make it possible to conduct this investigation into the diseases that cause health and economic burdens to Thais. Our group has long-term experiences in the study of genetics and molecular biology of diabetes, kidney diseases, and dengue virus infection in Thais. We therefore take this advantage to focus our research interest in the investigation of these diseases by applying advanced technologies in genetics, genomics, and molecular biology. The results of five projects studied are reported.

Project I: Genetics/Genomics and Molecular Biology of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. If it is untreated, DM will result in complications including retinopathy, nephropathy, neuropathy, and cardiovascular disease. Type 2 diabetes (T2D) is the most common, accounting for 90% of all DM patients. The monogenic T2D is classified as maturity-onset diabetes of the young (MODY), characterized by young age at onset with autosomal dominant inheritance. Identification of genes causing MODY and that influencing individual susceptibility to T2D leads to a better understanding of pathophysiology of diabetes. Our research group has shown that the six reported MODY genes account for a small proportion of MODY (19%) and early-onset T2D patients (10%) in Thais. We are the first group who identified the mutations and single nucleotide polymorphisms (SNPs) of *PAX4* caused MODY9 and T2D susceptibility, respectively. In the continuing studies, our group aimed to examine whether genetic variations of such strong candidate genes as *TCF7L2*, *CAPN10*, *AdipoQ*, and *PAX4* genes are associated with T2D in Thais or not. We found that these genes, especially *PAX4*, are associated with T2D in Thai patients. We studied into details of *AdipoQ* and *PAX4* variants identified in Thai patients and reported the abnormal structures and functions of mutant adiponectin and PAX4 proteins expressed in cultured cell lines. We also employed genomic approaches using DNA microarrays and exome sequencing to identify novel genes causing MODY and early-onset T2D in Thai families. We have discovered novel genes, which will be soon reported.

In addition, we also investigated the protective mechanism of sex hormones (estrogen and testosterone) against glucotoxicity on β-cells to understand the roles of these hormones in protection of DM and to search for an alternative strategy for prevention and treatment of DM, especially in elderly. We demonstrated that estrogen significantly decreases not only oxidative stress but also endoplasmic reticulum (ER) stress to protect against high glucose-induced pancreatic β-cell death. Similarly, testosterone can protect against male pancreatic β-cell apoptosis from glucotoxicity via reduction of both oxidative stress and ER stresses.

Project II: Genetics/Genomics and Molecular Biology of Kidney Stone Disease

Kidney stone disease (KSD) is an important public health problem in the Northeastern (NE) population of Thailand. Its prevalence is 5-10% with several thousands of new cases hospitalized for treatment each year. The etiology and pathogenesis of KSD in the NE Thai population are unknown but the disease in this population seems to be unique from what has been reported in other ethnic groups because it is not associated with the conditions of increased urinary stone promoters, such as hypercalciuria, hyperoxaluria, and hyperuricosuria. Our group has recently reported an initial evidence suggesting a genetic contribution to KSD in the NE Thai population because it was found to have familial aggregation with a high relative risk (λ_R = 3.18) among members of the affected families. To investigate into the role of genetic factor in pathogenesis of KSD in the NE Thai population, we have employed multiple genetic/genomic approaches, such as candidate-gene association study, genome-wide association study (GWAS), and genome-wide linkage analysis using DNA microarrays to identify disease-susceptibility and disease-causing genes. We firstly conducted a candidate gene association study and found the association between KSD in Northeastern Thai patients and *prothrombin* (*F2*) gene. After sequencing the entire coding regions of *F2*, we identified one exonic non-synonymous single nucleotide polymorphism (nsSNP; rs5896; c.494 C>T) in exon 6 resulting in a T165M substitution. Our results indicate that prothrombin variant (T165M) is associated with KSD risk in the NE Thai female patients. The genome-wide association study (GWAS) by using DNA microarrays was also conducted. A SNP rs759330, located at a predicted microRNA binding site at 3'UTR of *PAQR6* – a gene encoding progestin and adipoQ receptor family member VI, was found to be associated with KSD, suggesting that *PAQR6* is a modifying gene for KSD. We also selected a large family with KSD (UBRS082) to perform a genome-wide linkage and exome sequencing. KSD phenotype was inherited as autosomal dominant model in this family. Chromosomal regions with high logarithm of odd scores (LOD >2.80) were initially identified by genome-wide linkage and genetic variations in these regions were examined by exome sequencing. Two novel variations (p.N909K and p.K1809R) of *SCN10A* on chromosome 3, encoding Nav1.8α subunit of voltage-gated sodium channel, were co-segregated with KSD in this family without its presence in the normal control subjects. As these two variations were co-inherited in the same allele, they might have combined effects in causing KSD. An additional variation (p.V1149M) of *SCN10A* was identified in another affected family. Nav1.8α subunit mRNA and protein are expressed in human kidney tissues. All these findings provide evidences supporting that the mutations of *SCN10A* cause KSD in these families.

Project III: Molecular Mechanism of Human Kidney Anion Exchanger 1 Trafficking Associated with Distal Renal Tubular Acidosis

Human kidney anion exchanger 1 (kAE1) is a basolateral anion (Cl^-/HCO_3^-) exchanger of the acid-secreting type A intercalated cells in the distal nephron, involved in maintaining acid-base homeostasis in the human body. Several mutations in the *SLC4A1* gene encoding kAE1 result in autosomal dominant or autosomal recessive distal renal tubular acidosis (dRTA). This disease is characterized by an inability of the kidney to secrete H^+ into urine resulting in systemic metabolic acidosis often accompanied by several clinical manifestations including muscle weakness, growth retardation, metabolic bone disease, nephrocalcinosis, nephrolithiasis, chronic pyelonephritis, and renal failure. The *SLC4A1* mutations associated with dRTA usually do not cause defect in the anion exchange function of kAE1 but result in impaired trafficking or mistargeting of the mutant kAE1 proteins. However, it has yet been unknown how the protein trafficking fails or why mistargeting of kAE1 protein occurs. To understand the pathogenesis of

dRTA caused by *SLC4A1* mutations, it is necessary to investigate the trafficking and targeting process of kAE1 protein from its biosynthesis site to the cell surface in both normal and abnormal conditions.

Our group is interested in identifying the protein that interacts with kAE1 that plays a role in its basolateral trafficking. We identified kAE1-interacting proteins by using yeast two hybrid (Y2H) screening. The interaction between kAE1 and adaptor-related protein complex 1 mu1A (AP-1 mu1A), were identified. We have also discovered that kAE1 interacts with kinesin family member 3B (KIF3B) – a motor protein, in kidney cells, suggesting that KIF3B is involved in the trafficking of kAE1 to the plasma membrane of human kidney alpha-intercalated cells. However, it is not known how the intracellular sorting and trafficking of kAE1 from trans-Golgi network (TGN) to the basolateral membrane occur. We thus studied the role of basolateral-related sorting proteins, including mu1 subunit of adaptor protein (AP) complexes, clathrin, and protein kinase D, on kAE1 trafficking in polarized and non-polarized kidney cells. We found that AP-1 mu1A, AP-3 mu1, AP-4 mu1 and clathrin (but not AP-1 mu1B, PKD1 or PKD2) play crucial roles in intracellular sorting and trafficking of kAE1. We also demonstrated co-localization of kAE1 and basolateral-related sorting proteins in human kidney tissues by double immunofluorescence staining. These findings indicate that AP-1 mu1A, AP-3 mu1, AP-4 mu1, and clathrin are required for kAE1 sorting and trafficking from TGN to the basolateral membrane of acid-secreting alpha-intercalated cells.

Project IV: Molecular Pathogenesis of Dengue Virus Infected Liver Cells

Dengue virus (DENV) infection is one of the most important mosquito-borne viral diseases, affecting many million people worldwide. DENV particle contains a single positive-stranded RNA genome, encoding a single precursor polypeptide, which is cleaved by host and viral proteases into three structural proteins, including capsid (C), membrane (M), and envelope (E), and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Clinical symptoms of DENV infection range from a predominantly febrile disease, dengue fever (DF), to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which usually occurs in cases with subsequent infection with a different serotype of DENV. The patients with DHF generally present with hemorrhagic tendencies, plasma leakage, thrombocytopenia, and hemoconcentration. Liver cell injury is commonly observed in patients with DHF/DSS, as evident by elevation of aminotransferases, reactive hepatitis and fulminant hepatic failure. The cause of hepatocyte injury during DENV infection, which may lead to fulminant hepatic failure, remains unclear. In this project, we determined cell death responses and inflammatory cytokine production induced by DENV infection in cultured hepatic cells. And, the roles and mechanisms of DENV C and DENV NS5 proteins in cell death responses and induction of inflammatory cytokine were also investigated in the cultured hepatic cells. The results of these studies would provide insight into molecular pathogenesis of DENV infection causing liver cell injury and will facilitate the development of new therapeutic modalities for DENV infection.

We examined the expression of cell death genes during DENV-infection of HepG2 cells by using real-time PCR arrays. The expression changes were consistent with activation of apoptosis and autophagy, including the up-regulation of *RIPK2*, *HRK*, *TGF*-β, *PERK*, and *LC3B*. RIPK2 – receptor-interacting serine/threonine protein kinase 2 is a crucial mediator of multiple stress responses that leads to the activation of caspase, NF-κB and MAP kinases including JNK and p38. The inhibition of RIPK2 expression by SB203580 significantly reduced apoptosis and suppression of endogenous RIPK2 in DENV-infected HepG2 cells by small interfering RNA (siRNA) significantly decreased apoptosis, suggesting for the first time that RIPK2 plays a role in DENV-mediated apoptosis. From real-time PCR arrays, we also found the up-regulation of *cathepsin* gene expression in DENV-infected HepG2 cells.

Cathepsins – cysteine proteases inside the lysosome were previously reported to be up-regulated in patients with DHF. We showed for the first time that DENV induces lysosomal membrane permeabilization. The resulting cytosolic cathepsin B and S contributed to apoptosis via caspase-9 and caspase-3 activation, which was significantly reduced by cathepsin B or S inhibitors and cathepsin B-siRNA.

We have previously described the translocation of DENV C into nucleus and its interaction with death-domain-associate (DAXX) protein to induce apoptosis. Expression of CD137, which is a member of the tumor necrosis factor receptor family, increased significantly in HepG2 cells expressing DENV C. CD137 recruits TNF receptor associated factor 2 (TRAF2) and activates apoptosis signal regulating kinase 1 (ASK1), resulting in activation of cJun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). p38 MAPK participates in both apoptosis-related signaling and pro-inflammatory cytokine production. The role of p38 MAPK in DENV-infected HepG2 cells was examined using siRNA, which showed that DENV infection activated p38 MAPK and induced apoptosis. Thus, DENV induces CD137 signaling to enhance apoptosis by increasing TNFα production via activation of p38 MAPK.

The *in vivo* role of ERK1/2, a member of the MAPK family, in a mouse model of DENV infection was also examined. Our results showed that DENV induces phosphorylation of ERK1/2 and increases apoptosis. Inhibition of phosphorylated ERK1/2 by the selective ERK1/2 inhibitor, FR180204, limits hepatocyte apoptosis and reduces DENV-induced liver injury. Clinical parameters, including leucopenia, thrombocytopenia, transaminases and histology, show improvements after FR180204 treatment. Caspase-3 was significantly decreased in FR180204 treated DENV-infected mice compared to the levels of untreated DENV-infected mice, suggesting the role of ERK1/2 signaling in immune-mediated liver injury during DENV infection.

DAXX was also identified to interact with DENV NS5 by yeast two-hybrid (Y2H) assay. The *in vivo* relevance of this interaction was suggested by co-immunoprecipitation and nuclear co-localization of these two proteins in HEK293 cells expressing DENV NS5. HEK293 cells expressing DENV NS5-K/A, which were mutated at the nuclear localization sequences (NLS), were created to assess its functional roles in nuclear translocation, DAXX interaction, and cytokine production. In the absence of NLS, DENV NS5 could neither translocate into the nucleus nor interact with DAXX to increase the DHF-associated cytokine, RANTES (CCL5) production. This demonstrates the interaction between DENV NS5 and DAXX and the role of the interaction on the modulation of RANTES production.

Increased levels of cytokines - the so-called 'cytokine storm', contribute to the pathogenesis of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). We therefore compared the expression of cytokine genes between mock-infected and DENV-infected HepG2 cells using a real-time PCR array and revealed several up-regulated chemokines and cytokines, including CXCL10 and TNF-α. In this study, we also used compound A (CpdA), a plant-derived phenyl aziridine precursor containing anti-inflammatory action and acting as a dissociated nonsteroidal glucocorticoid receptor modulator, as a candidate agent to modulate secretion of DENV-induced cytokines. CpdA is not a glucocorticoid but has an anti-inflammatory effect with no metabolic side effects as steroidal ligands. CpdA significantly reduced DENV-induced CXCL10 and TNF-α secretion and decreased leukocyte migration, indicating for the first time the therapeutic potential of CpdA in decreasing massive immune activation during DENV infection.

Project V: Production of Human Single Chain Antibody Variable Fragments and Peptide Inhibitors Specific to Dengue Virus Proteins

Nowadays, a licensed vaccine and anti-viral agent for DENV infection have not been available and only supportive treatment is given to the patients. We proposed that blocking or inhibiting functions of viral proteins could reduce disease severity and symptoms in the DENV-infected patients. In this project, we produced human single chain antibody variable fragments (HuScFv) specific to dengue virus proteins and test their binding and inhibiting activities to the corresponding antigens with an ultimate objective to produce therapeutic biomolecules for treatment of dengue virus infection.

HuScFv molecules were screened and selected from the human antibody phage display library by using purified recombinant DENV NS1 (rNS1), full-length envelope (rFL-E) and its domain III (rEDIII) proteins as target antigens for bio-panning. HuScFv from two phagemid transformed *E. coli* clones, i.e., clones 11 and 13, bound to the rNS1 as well as native NS1 in both secreted and intracellular forms. Culture fluids of the HuScFv11/HuScFv13 exposed DENV2 infected cells had significant reduction of the infectious viral particles, implying that the antibody fragments affected the virus morphogenesis or release. rEDIII-specific HuScFv15A exhibited neutralizing effect to DENV infection in Vero cells in a dose-dependent manner as determined by plaque formation and cell ELISA. Epitope mapping and molecular docking results concordantly revealed interaction of HuScFv15A to functional loop structure in EDIII of the DENV E protein. Although the functions of the epitopes and the molecular mechanism of the HuScFvs further investigations, these small antibodies have high potential for development as an effective anti-DENV biomolecules.

Furthermore, we used molecular docking to search for a safe anti-DENV drug. The short peptides targeting to the hydrophobic pocket on DENV E protein; a structural transition in the membrane fusion in DENV infection process, were identified. The information of predicted ligand-binding site of reported active compounds to DENV2 hydrophobic pocket was also used for peptide inhibitor selection. The di-peptide, EF, was the most effective on DENV2 infection inhibition *in vitro* with a half maximal inhibition concentration (IC50) of 96 μM. Treatment of DENV2 with EF at the concentration of 200 μM resulted in 83.47% and 84.15% reduction of viral genome and intracellular E protein, respectively. Among four DENV serotypes, DENV2 was the most effective for the inhibition. Our results provide the proof-of-concept for development of therapeutic peptide inhibitors against DENV infection by the computer-aided molecular design.

Keywords: genomics; molecular biology; diabetes mellitus; kidney stone; distal renal tubular acidosis; dengue virus; pathogenesis; human single chain antibody variable fragments (HuScFv); peptide inhibitors

บทคัดย่อ

ความผันแปรของจีโนมมนุษย์ นอกจากจะเป็นสาเหตุให้เกิดโรคในมนุษย์ได้โดยตรงแล้ว ยังส่งผลกระทบต่อการเกิดโรคในขั้นตอนต่างๆ ตั้งแต่การทำให้เกิดความเสี่ยง การมีผลต่อกระบวนการทางพยาธิวิทยา การมีผลต่อการดำเนินของโรคและพัฒนาสู่ความรุนแรง ตลอดจนการตอบสนองต่อการรักษาและการหายจากความเจ็บป่วย ในปัจจุบันเป็นที่ทราบกันแล้วว่า โรคชนิดเดียวกัน อาจจะมีสาเหตุและกระบวนการเกิดโรคที่แตกต่างกัน เนื่องจากความแตกต่างทางพันธุกรรมของประชากรและของบุคคล มีหลักฐานที่เชื่อถือได้ว่า การกลายพันธ์ของยีนชนิดที่รุนแรง แต่พบน้อย เป็นสาเหตุของโรคที่ซับซ้อนของมนุษย์ และความหลากหลายทางพันธุกรรม มีผลต่อการเกิดโรคในขั้นตอนต่างๆ ดังนั้นโดยอาศัยข้อมูลที่กล่าวมานี้เป็นฐาน คณะผู้วิจัย จึงตั้งสมมติฐานว่า โรคที่ซับซ้อนในคนไทยเกิดจากการกลายพันธุ์ของยีนที่รุนแรง แต่พบน้อย ซึ่งมีความหลากหลาย ดังนั้น จึงมีความจำเป็นที่จะต้องศึกษาสาเหตุและพยาธิกำเนิดของโรคในคนไทยโดยตรง เพื่อที่ให้เข้าใจธรรมชาติและการหาวิธีการรักษาที่เหมาะสม การมีข้อมูลจีโนมมนุษย์และเทคนิคทางอณูชีววิทยาที่ทรงพลังช่วยให้มีความเป็นไปได้ในการศึกษาโรค ซึ่งมีความสำคัญทางสาธารณสุขและมีผลกระทบทางเศรษฐกิจของคนไทย คณะผู้วิจัยมีประสบการณ์ที่ยาวนาน ในการศึกษาทางพันธุศาสตร์และอณูชีววิทยาของโรคเบาหวาน โรคไต และโรคไข้เลือดออก ในคนไทย จึงอาศัยประโยชน์ในข้อนี้ ที่จะมุ่งความสนใจในการศึกษาโรคเหล่านี้ต่อไป โดยใช้เทคโนโลยีที่ก้าวหน้าทางด้านพันธุศาสตร์ จีโนมิกส์ และอณูชีววิทยา ผลการศึกษา ซึ่งแบ่งเป็น 5 โครงการ มีดังต่อไปนี้

โครงการที่ 1: อณูพันธุศาสตร์/จีโนมิกส์ และอณูชีววิทยาของโรคเบาหวาน

โรคเบาหวานเป็นโรคเรื้อรังทางเมตาบอลิค ทำให้เกิดภาวะน้ำตาลในเลือดสูง โรคนี้หากไม่มีการรักษา จะทำให้เกิดภาวะแทรกซ้อนที่สำคัญ คือ จอตาเสื่อม ไตเสื่อม ระบบประสาทถูกทำลาย และเกิดความผิดปกติที่หลอดเลือดและหัวใจ โรคเบาหวานชนิดที่ 2 เป็นชนิดที่พบบ่อยที่สุด มีสัดส่วนร้อยละ 90 ของโรคเบาหวานทั้งหมด โรคเบาหวานชนิดที่ 2 ซึ่งเกิดจากยีนเดี่ยว ในกลุ่มที่เรียกว่า โรคเบาหวานชนิดที่ 2 ในผู้ที่มีอายุน้อย (MODY) มีการถ่ายทอดแบบลักษณะเด่น การศึกษายีนซึ่งเป็นสาเหตุของ MODY และความเกี่ยวข้องกับการเกิดโรคเบาหวานชนิดที่ 2 จะช่วยให้เข้าใจพยาธิสรีรวิทยาของโรคเบาหวานได้ดียิ่งขึ้น คณะผู้วิจัยได้เคยรายงานว่า ยีนซึ่งเคยมีรายงานมาก่อนว่าเป็นสาเหตุของ MODY ในประชากรอื่น จำนวน 6 ยีน เป็นสาเหตุในสัดส่วนที่น้อยของ MODY (ร้อยละ 18) และโรคเบาหวานชนิดที่ 2 ในผู้ป่วยที่มีอายุน้อย (ร้อยละ 10) ในคนไทย คณะผู้วิจัยเป็นกลุ่มแรกที่ค้นพบการกลายพันธุ์และความผันแปรของนิว-คลีโอไทด์ (SNP) ของยีน *PAX4* ว่าเป็นสาเหตุของ MODY9 และการเกิดความเสี่ยงในการเกิดโรคเบาหวานชนิดที่ 2 (ตามลำดับ) ในโครงการวิจัยต่อเนื่องนี้ คณะผู้วิจัยได้ทำการศึกษาความผันแปรทางพันธุกรรมของยีนที่มีการรายงานอย่างชัดเจนว่าทำให้เกิดโรคเบาหวาน คือ ยีน *TCF7L2*, *CAPN10*, *AdipoQ* และ *PAX4* ว่าเกี่ยวข้องกับการเกิดโรคเบาหวานในคนไทยหรือไม่ คณะผู้วิจัยได้พบว่ายีนเหล่านี้ โดยเฉพาะยีน *PAX4* เกี่ยวข้องกับการเกิดโรคเบาหวานในคนไทย คณะผู้วิจัยได้ศึกษาในรายละเอียดของ ความผันแปรของยีน *AdipoQ* และ *PAX4* ที่ค้นพบในคนไทย และได้รายงานความผิดปกติทางโครงสร้างและการทำหน้าที่ของของโปรตีน adiponectin และโปรตีน PAX4 ที่ผันแปรไป โดยการทำให้มีการสังเคราะห์โปรตีนในเซลล์เพาะเลี้ยง นอกจากนี้ คณะผู้วิจัยยังได้ใช้วิธีทางจีโนมิกส์ โดยวิธีดีเอ็นเอไมโครอะเรย์ (DNA microarrays) และวิธีวิเคราะห์ลำดับนิวคลีโอไทด์ของเอ็กโซม (exome sequencing) เพื่อค้นหายีนใหม่ที่ทำให้เกิด MODY และโรคเบาหวานชนิดที่ 2 ในผู้ที่อายุน้อย ในครอบครัวผู้ป่วยไทย คณะผู้วิจัยได้ค้นพบยีนใหม่ ซึ่งกำลังจะตีพิมพ์เพื่อรายงานต่อไป

นอกจากนี้ คณะผู้วิจัยได้ทำการศึกษากลไกการป้องกันภาวะเป็นพิษจากน้ำตาลสูง ต่อเบต้า-เซลล์ของฮอร์โมนเพศ (เอสโตรเจนและเทสโทสเตอโรน) เพื่อให้เข้าใจบทบาทของฮอร์โมนเหล่านี้ ต่อการป้องการโรคเบาหวานและการค้นหาทางเลือกในการป้องกันและรักษาโรคเบาหวาน โดยเฉพาะในผู้สูงอายุ คณะผู้วิจัยได้แสดงให้เห็นว่าเอสโตรเจนสามารถลด ทั้งภาวะ oxidative stress และภาวะ endoplasmic reticulum (ER) stress ซึ่งจะป้องกันการตายของเบต้า-เซลล์ได้ ในทำนองเดียวกัน เทสโทสเตอโรนก็สามารถป้องกันการตายของเบต้า-เซลล์ของเพศชาย จากภาวะน้ำตาลสูงได้ โดยการลดทั้งภาวะ oxidative stress และ ER stress

โครงการที่ 2: อณูพันธุศาสตร์/จีโนมิกส์ และอณูชีววิทยาของโรคนิ่วไต

โรคนิ่วไตเป็นปัญหาสาธารณสุขสำคัญในประชากรภาคอิสานของไทย มีอัตราความชุกร้อยละ 5-10 และมีผู้ป่วยใหม่เข้ารับการรักษาในโรงพยาบาล ปีละหลายพันคน สาเหตุและพยาธิกำเนิดของโรคนิ่วไตในประชากรภาคอิสานของไทย ยังไม่เป็นที่ทราบแน่ชัด แต่น่าจะมีความแตกต่างจากโรคนิ่วไตที่เคยรายงานในประชากรอื่น เพราะไม่พบภาวะช่วยส่งเสริมการเกิดโรคนิ่ว เช่น ภาวะแคลเซียมสูงในปัสสาวะ ภาวะอ๊อกซาเลตสูงในปัสสาวะ และภาวะกรดยูลิคสูงในปัสสาวะ เป็นต้น คณะผู้วิจัยได้รายงานเมื่อเร็วๆ นี้ว่า มีหลักฐานแสดงถึงความเกี่ยวข้องของปัจจัยทางพันธุกรรมกับการเกิดโรคนิ่วไตในประชากรภาคอิสานของไทย เพราะพบว่ามีผู้ป่วยหลายคนมาจากครอบครัวเดียวกัน และสมาชิกจากครอบครัวของผู้ป่วยจะมีความเสี่ยงในการเกิดโรคมากกว่าประชากรทั่วไปประมาณ 3 เท่า เพื่อที่จะศึกษาบทบาทของปัจจัยทางพันธุกรรมต่อพยาธิกำเนิดของโรคนิ่วไตในประชากรภาคอิสานของไทย คณะผู้วิจัยได้ใช้วิธีทางพันธุศาสตร์และจีโนมิกส์หลายวิธี เช่น candidate-gene association study, genome-wide association study (GWAS), และ genome-wide linkage analysis โดยใช้ DNA microarrays เพื่อค้นหายีนที่ช่วยส่งเสริม และยีนที่เป็นสาเหตุของโรค ในเบื้องต้น คณะผู้วิจัยได้พบความเกี่ยวข้องของยีน *prothrombin* (*F2*) กับการช่วยส่งเสริมเกิดโรคนิ่วไต จึงวิเคราะห์ลำดับนิวคลีโอไทด์ของยีนนี้ทั้งยีน และพบว่าการเปลี่ยนกรดอะมิโนที่ตำแหน่ง 165 ของโปรตีนจาก threonine เป็น methionine (T165M) มีผลช่วยส่งเสริมการเกิดโรคนิ่วไตในผู้หญิงภาคอิสานของไทย โดยการศึกษาด้วยวิธี GWAS คณะผู้วิจัยพบว่า SNP rs759330 ซึ่งเป็นตำแหน่งจับของไมโครอาร์เอ็นเอ (micro RNA) บน 3' UTR ของยีน *PAQR6* ซึ่งเป็นยีนควบคุมการสร้างตัวรับของฮอร์โมนโปรเจสติน (progestin) และ adipoQ receptor family member VI มีความเกี่ยวข้องกับโรคนิ่วไต โดยมีผลเปลี่ยนแปลงต่อการเกิดโรค (modifying gene) คณะผู้วิจัยจึงคัดเลือกครอบครัวของผู้ป่วยโรคนิ่วไตที่มีขนาดใหญ่ (UBRS082) ซึ่งมีการถ่ายทอดของโรคแบบลักษณะเด่น เพื่อทำการศึกษาโดยวิธี genome-wide linkage และ exome sequencing โดยในเบื้องต้น คณะผู้วิจัยได้ใช้วิธี genome-wide linkage เพื่อวิเคราะห์หาบริเวณบนโครโมโซมที่น่าจะมียีนก่อโรค ก่อน โดยทำการคำนวณค่า logarithm of odd scores (LOD) ซึ่งได้ค่า LOD >2.80 ในบางบริเวณ ต่อมา จึงตรวจหาความผันแปรทางพันธุกรรมด้วยวิธี exome sequencing ผลการศึกษาพบความผันแปรของยีน *SCN10A* บนโครโมโซมคู่ที่ 3 ทำให้มีการเปลี่ยนชนิดของกรดอะมิโนในโปรตีน Nav1.8α subunit of voltage-gated sodium channel สองตำแหน่ง คือ p.N909K and p.K1809R ซึ่งถ่ายทอดร่วมกันและถ่ายทอดร่วมกับโรคนิ่วไตในครอบครัวนี้ ซึ่งอาจจะแสดงถึงผลในการก่อโรคร่วมกัน จากการศึกษาเพิ่มเติม คณะผู้วิจัยได้พบความผันแปรของยีนเดียวกัน ทำให้มีการเปลี่ยนชนิดของกรดอะมิโนอีกหนึ่งตำแหน่ง คือ p.V1149M ในผู้ป่วยโรคนิ่วไตอีกครอบครัวหนึ่ง และเมื่อทำการศึกษาการแสดงออกของยีนนี้ในไตของมนุษย์ ก็พบว่ามีการแสดงออกของเอ็ม-อาร์เอ็นเอ (mRNA) และโปรตีนในเนื้อไตของมนุษย์ จากผลการศึกษาทั้งหมด จึงสรุปได้ว่าการกลายพันธุ์ของยีน *SCN10A* ทำให้เกิดโรคนิ่วไตในครอบครัวที่ศึกษานี้

โครงการที่ 3: กลไกในระดับอณูของการเคลื่อนย้ายโปรตีนแอนไอออน เอ็กเช็นเจอร์-วัน ซึ่งเกี่ยวข้องกับโรคไตผิดปกติในการขับกรด

โปรตีนแอนไอออน เอ็กเช็นเจอร์-วันในไตมนุษย์ (kAE1) ทำหน้าที่แลกเปลี่ยนแอนไอออน (Cl^-/HCO_3^-) บริเวณด้านฐานของเยื่อหุ้มเซลล์ (basolateral membrane) ของเซลล์ขับกรด ซึ่งอยู่ในบริเวณดีส-ทอลแนบฟอน (distal nephron) ซึ่งมีความสำคัญในการควบคุมภาวะสมดุลย์กรด-ด่างของร่างกายมนุษย์ การกลายพันธุ์ในยีน *SLC4A1* ซึ่งควบคุมการสร้างโปรตีน kAE1 ทำให้เกิดโรคไตผิดปกติในการขับกรด (distal renal tubular acidosis หรือ dRTA) ซึ่งมีการถ่ายทอดได้ทั้งแบบลักษณะเด่นและแบบลักษณะด้อย โรคนี้เกิดจากไตไม่สามารถจะขับไฮโดรเจนไอออน (H^+) ออกทางปัสสาวะได้ ทำให้เกิดภาวะเป็นกรดในร่างกาย ซึ่งส่งผลให้เกิดอาการรุนแรงหลายอย่าง ได้แก่ กล้ามเนื้ออ่อนแรง เจริญเติบโตช้า กระดูกอ่อน เกิดนิ่วและการสะสมของแคลเซียมในไต กรวยไตอักเสบ และภาวะไตวาย การกลายพันธุ์ของยีน *SLC4A1* ซึ่งทำให้เกิดโรคไตผิดปกติในการขับกรด มักจะไม่ได้ทำให้โปรตีน kAE1 สูญเสียหน้าที่ในการแลกเปลี่ยนแอนไอออน แต่ทำให้โปรตีนผิดปกติในการเคลื่อนย้ายหรืออยู่ผิดตำแหน่ง แต่ยังไม่เป็นที่ทราบอย่างแน่ชัดว่า โปรตีน kAE1 ผิดปกติในการเคลื่อนย้ายหรืออยู่ผิดที่ได้อย่างไร ดังนั้น เพื่อที่จะเข้าใจพยาธิกำเนิดของโรคไตผิดปกติในการขับกรดจากการกลายพันธุ์ของยีน *SLC4A1* จึงจำเป็นจะต้องศึกษาให้เข้าใจกระบวน-การเคลื่อนย้ายของโปรตีน kAE1 จากแหล่งที่มีการสร้างไปสู่ผิวเซลล์ ทั้งในภาวะปกติและผิดปกติ

คณะผู้วิจัยมีความสนใจในการค้นหาโปรตีน ซึ่งมีปฏิสัมพันธ์กับโปรตีน kAE1 และมีบทบาทในการเคลื่อนย้ายโปรตีน kAE1 ไปที่เยื่อหุ้มเซลล์ในด้านฐานของเซลล์ (basolateral membrane) โดยใช้วิธี yeast two hybrid (Y2H) ทำให้ค้นพบว่าโปรตีน kAE1 มีปฏิสัมพันธ์กับโปรตีน adaptor-related protein complex 1 mu1A (AP-1 mu1A) นอกจากนี้ยังพบว่าโปรตีน kAE1 มีปฏิสัมพันธ์กับโปรตีน kinesin family member 3B (KIF3B) ซึ่งเกี่ยวข้องกับการขนส่งโปรตีน kAE1 ไปยังเยื่อหุ้มเซลล์ของเซลล์ขับกรดในไต แต่เนื่องจากยังไม่เป็นที่ทราบว่าโปรตีน kAE1 ถูกแยกภายในเซลล์ (intracellular sorting) และเคลื่อนย้าย (trafficking) จาก trans-Golgi network (TGN) ไปสู่เยื่อหุ้มเซลล์ด้านฐานของเซลล์ได้อย่างไร คณะผู้วิจัยจึงทำการศึกษาบทบาทของโปรตีนที่เกี่ยวข้องในการแยกโปรตีน kAE1 จาก TGN ไปสู่เยื่อหุ้มเซลล์ และพบว่าโปรตีนที่เกี่ยวข้อง ได้แก่ AP-1 mu1A, AP-3 mu1, AP-4 mu1 และ clathrin (ส่วนโปรตีนที่ไม่เกี่ยวข้อง ได้แก่ AP-1 mu1B, PKD1 และ PKD2) และพบว่าโปรตีนเหล่านี้ถูกย้อมอยู่ในตำแหน่งเดียวกันกับโปรตีน kAE1 ในชิ้นเนื้อจากไตของมนุษย์ ผลการศึกษานี้แสดงว่าโปรตีนดังกล่าว มีบทบาทในการแยกและเคลื่อนย้ายโปรตีน kAE1 จาก TGN ไปสู่เยื่อหุ้มเซลล์ด้านฐานของเซลล์ขับกรดในไตมนุษย์

โครงการที่ 4: พยาธิกำเนิดระดับอณูของเซลล์ตับซึ่งติดเชื้อไวรัสเด็งกี

การติดเชื้อไวรัสเด็งกีเป็นปัญหาที่สำคัญของโรคที่เกิดจากไวรัสซึ่งมียุงเป็นพาหะ เพราะมีผู้ติดเชื้อจำนวนหลายล้านคนทั่วโลก อนุภาคไวรัสเด็งกีประกอบด้วยจีโนมซึ่งเป็นอาร์เอ็นเอสายบวก ใช้ในการถอดรหัสเพื่อสังเคราะห์โปรตีนโครงสร้าง 3 ชนิด ได้แก่ capsid (C), membrane (M), และ envelope (E) และโปรตีนที่ไม่ใช่โครงสร้างอีก 7 ชนิด ได้แก่ NS1, NS2A, NS2B, NS3, NS4A, NS4B และ NS5 อาการของการติดเชื้อไวรัสเด็งกีมีตั้งแต่เป็นไข้ ไข้เลือดออก และกลุ่มอาการช็อกจากการติดเชื้อไวรัสนี้ ซึ่งอาการที่รุนแรง มักจะเกิดกับผู้ป่วยที่ติดเชื้อไวรัสซ้ำ แต่เป็นคนละซีโรทัยป์กับการติดเชื้อครั้งก่อน ผู้ป่วยที่เป็นไข้เลือดออกมักจะมีอาการเลือดออกง่าย มีการรั่วของสารน้ำออกนอกหลอดเลือด เกล็ดเลือดต่ำ และมีเลือดเข้มข้น ในผู้ป่วยที่มีอาการไข้เลือดออกและช็อกมักจะพบว่ามีการทำลายของเซลล์ตับ เนื่องจากพบว่ามีเอ็นไซม์ aminotransferases สูงขึ้น มีตับอักเสบ และมีภาวะตับล้มเหลวเฉียบพลัน สาเหตุของการทำลายเซลล์ตับในขณะที่มีการติดเชื้อไวรัสเด็งกี ซึ่งอาจจะนำไปสู่ภาวะตับล้มเหลวเฉียบพลัน ยัง

ไม่เป็นที่ทราบแน่ชัด ในโครงการนี้ คณะผู้วิจัยได้ศึกษาการตอบสนองซึ่งนำไปสู่การตายของเซลล์ และการสร้างสารซัยโตไคน์ ซึ่งทำให้เกิดการอักเสบ ในเซลล์ตับที่นำมาเพาะเลี้ยงและทำให้ติดเชื้อไวรัสเด็งกี นอกจากนี้ยังศึกษาบทบาทและกลไกที่โปรตีน capsid และ NS5 ซึ่งเกี่ยวข้องกับการตอบสนองทั้งสองอย่างนี้ด้วย ผลการศึกษานี้จะทำให้เข้าใจพยาธิกำเนิดในระดับอณูของการติดเชื้อเด็งกี ซึ่งให้เกิดการทำลายของเซลล์ตับ และจะช่วยในการพัฒนารูปแบบการรักษาใหม่จากการติดเชื้อไวรัสเด็งกี

คณะผู้วิจัยได้ศึกษาการแสดงออกของยีนที่เกี่ยวข้องกับการตายของเซลล์ระหว่างการติดเชื้อไวรัสเด็งกี โดยใช้เซลล์ HepG2 และวิธี real-time PCR arrays ผลการศึกษาพบว่ามีการกระตุ้นยีนที่เกี่ยวข้องกับการตายแบบอะพอพโตซีส (apoptosis) และการเกิดออโตฟาจี (autophagy) ยีนที่มีการแสดงออกเพิ่มขึ้นได้แก่ *RIPK2*, *HRK*, *TGF*-β, *PERK* และ *LC3B* โปรตีน RIPK2 เป็นเอ็นไซม์ไคแนส (kinase) ที่มีปฏิสัมพันธ์กับโปรตีนตัวรับ และมีบทบาทสำคัญต่อการตอบสนองหลายอย่าง ซึ่งทำให้เกิดการกระตุ้นเอ็นไซม์คาสเปส (caspase) โปรตีน NF-kB และเอ็นไซม์ MAP kinases ได้แก่ JNK และ p38 การยับยั้ง RIPK2 ด้วยสารยับยั้ง SB203580 ทำให้ลดการตายแบบอะพอพโตซีสของเซลล์ และการลด RIPK2 โดยใช้ siRNA ในเซลล์ HepG2 ที่ติดเชื้อไวรัสเด็งกี ทำให้ลดการตายแบบอะพอพโตซีสได้อย่างมีนัยสำคัญ แสดงว่า RIPK2 มีบทบาทในการต่อการตายแบบอะพอพโตซีสของเซล์ที่ติดเชื้อไวรัสเด็งกี นอกจากนี้การศึกษาด้วยวิธี real-time PCR arrays คณะผู้วิจัยยังพบว่ามีการเพิ่มการแสดงออกของยีน *cathepsin* ในเซลล์ HepG2 ที่ติดเชื้อไวรัสเด็งกี cathepsins เป็น cysteine proteases ภายในไรโซโซม (lysosome) ซึ่งเคยมีรายงานว่ามีการเพิ่มขึ้นในผู้ป่วยไข้เลือดออกจากการติดเชื้อไวรัสเด็งกี คณะผู้วิจัยได้แสดงให้เห็นเป็นครั้งแรกว่า ไวรัสเด็งกีทำให้เยื่อหุ้มไรโซโซมสูญเสียคุณสมบัติ เมื่อ cathepsin B และ S ออกสู่ซัยโตพลาซึม จะทำให้เซลล์ตายแบบอะพอพโตซีส ผ่านทางการกระตุ้น caspase-9 และ caspase-3 ซึ่งสามารทำให้การตายแบบนี้ลดลงได้ โดยการใช้สารยับยั้งต่อ cathepsin B และ S และการใช้ cathepsin B-siRNA

คณะผู้วิจัยได้เคยรายงาน การเคลื่อนที่เข้าสู่นิวเคลียสของโปรตีนแค็บซีส (capsid protein) จากไวรัสเด็งกี และปฏิสัมพันธ์ระหว่างโปรตีนแค็บซีสกับโปรตีน DAXX ซึ่งนำไปสู่การตายของเซลล์แบบอะพอพโตซีส ในเซลล์ HepG2 ซึ่งทำให้มีการสร้างโปรตีนแค็บซีสของไวรัสเด็งกี จะมีการแสดงออกเพิ่มขึ้นของโปรตีน CD137 ซึ่งเป็นสมาชิกของ tumor necrosis factor receptor family โปรตีน CD137 จะดึงโปรตีน TNF receptor associated factor 2 (TRAF2) ให้เข้ามารวม และกระตุ้นโปรตีน apoptosis signal regulating kinase 1 (ASK1) ส่งผลทำให้เกิดการกระตุ้น cJun N-terminal kinase (JNK) และโปรตีน p38 mitogen-activated protein kinase (MAPK) โปรตีน p38 MAPK มีส่วนเกี่ยวข้องกับทั้งการตายแบบอะพอพโตซีส และการสร้างสารซัยโตไคน์ซึ่งกระตุ้นการอักเสบ บทบาทของโปรตีน p38 MAPK ในเซลล์ HepG2 ที่ติดเชื้อไวรัสเด็งกี ได้มีการศึกษาโดยใช้ siRNA ซึ่งผลแสดงว่าการติดเชื้อไวรัสเด็งกีทำให้มีการกระตุ้น p38 MAPK และเหนี่ยวนำให้เกิดการตายแบบอะพอพโตซีส จึงสรุปได้ว่าไวรัสเด็งกีทำให้เกิดการตายของเซลล์แบบอะพอพโตซีส โดยมีการเหนียวนำสัญญาณผ่านทางโปรตีน CD137 ทำให้มีการผลิต TNF-α มากขึ้น โดยผ่านการกระตุ้นโปรตีน p38 MAPK

คณะผู้วิจัยได้ศึกษาบทบาทของ ERK1/2 ซึ่งเป็นสมาชิกหนึ่งของ MAPK family ในสัตว์ทดลองโดยใช้หนูที่ทำให้ติดเชื้อไวรัสเด็งกี ผลการทดลองพบว่าไวรัสเด็งกีเหนี่ยวนำการเกิดการเติมหมู่ฟอสเฟต (phosphorylation) ของ ERK1/2 และทำให้เซลล์มีการตายแบบอะพอพโตซีสเพิ่มขึ้น เมื่อทดลองใช้สารยับยั้งการเติมหมู่ฟอสเฟตบน ERK1/2 ที่จำเพาะ คือ FR180204 สามารถทำให้ลดการตายของเซลล์ตับแบบอะพอพโตซีสลงและลดการทำลายของตับจากการติดเชื้อไวรัสเด็งกีได้ นอกจากนี้การใช้ FR180204 ยังทำให้ค่าผลการตรวจซึ่งมีความสำคัญทางคลินิก ได้แก่ leucopenia, thrombocytopenia, transaminases และ histology เปลี่ยนแปลงไปในทางที่ดีขึ้น ในหนูที่ติดเชื้อไวรัสเด็งกีและให้ FR180204 ทำให้มีการ

ลดลงของ caspase-3 แสดงว่า ERK1/2 มีบทบาทในการสื่อสัญญาทำให้เกิดการทำลายเซลล์ตับในระหว่างที่ติดเชื้อไวรัสเด็งกี

คณะผู้วิจัยได้พบว่าโปรตีน DAXX จับกับโปรตีน NS5 ของไวรัสเด็งกี โดยการศึกษาด้วยวิธี yeast two-hybrid (Y2H) ซึ่งได้ทำการศึกษาเพื่อยืนยันผลในเซลล์ HEK293 ด้วยวิธี co-immunoprecipitation และ nuclear co-localization การจับกันระหว่างโปรตีน DAXX และโปรตีน NS5 ในนิวเคลียสทำให้มีการกระตุ้นการสร้างซัยโตไคน์ RANTES (CCL5) หากทำการเปลี่ยนกรดอะมิโนของ NS5 บริเวณ nuclear localization sequence (NLS) จะทำให้เข้านิวเคลียสไม่ได้ ไม่มีการจับกับ DAXX และไม่กระตุ้นการสร้างสารซัยโตไคน์ RANTES ผลการทดลองนี้แสดงว่าการจับกันระหว่างโปรตีน NS5 ของเด็งกีและ DAXX มีบทบาทในการควบคุมการสร้างสารซัยโตไคน์ RANTES

การเพิ่มขึ้นของสารซัยโตไคน์ จนเกิดภาวะ “พายุซัยโตไคน์ (cytokine storm)” ทำให้เกิดโรคไข้เลือดออกและภาวะช็อกในผู้ป่วยที่ติดเชื้อไวรัสเด็งกี คณะผู้วิจัยได้ศึกษาเปรียบเทียบ การแสดงออกของยีนซัยโตไคน์ในเซลล์ที่ไม่ติดเชื้อและที่ติดเชื้อไวรัสเด็งกี และพบว่ามีการเพิ่มขึ้นของซัยโตไคน์หลายชนิด เช่น *CXCL10* และ *TNF*-α คณะผู้วิจัยได้ทดลองใช้สารคอมเปาวด์-เอ (CpdA) ซึ่งมีธรรมชาติเป็นสาร phenyl aziridine precursor ที่สกัดมาจากพืชทะเลทราย และมีฤทธิ์ยับยั้งการอักเสบ โดยสามารถจับได้กับ glucocorticoid receptor แต่ไม่มีการกระตุ้นยีนที่จะก่อให้เกิดผลข้างเคียง (side effects) เหมือน glucocorticoid ผลการทดลองปรากฏว่า CpdA สามารถลดการหลั่งสารซัยโตไคน์ CXCL10 และ TNF-α และสามารถลดการเคลื่อนที่ของเม็ดเลือดขาวได้ ซึ่งเป็นการแสดงครั้งแรกว่า CpdA สามารถใช้ลดการกระตุ้นระบบภูมิคุ้มกันอย่างรุนแรงในภาวะที่ติดเชื้อไวรัสเด็งกีได้

โครงการที่ 5: การผลิตแอนติบอดีสายเดี่ยวของมนุษย์ และเปปไทด์ยับยั้งที่จำเพาะต่อโปรตีนของไวรัสเด็งกี

ในปัจจุบัน ยังไม่มีวัคซีนที่ได้รับการจดทะเบียนและยังไม่มียาต้านไวรัสเด็งกี การดูแลรักษาผู้ป่วยยังเป็นการดูแลรักษาแบบประคับประคอง คณะผู้วิจัยจึงตั้งสมมุติฐานว่า การปิดกั้นหรือยับยั้งการทำงานของโปรตีนของไวรัส สามารถลดความรุนแรงและอาการของผู้ป่วยที่ติดเชื้อไวรัสเด็งกีได้ ในโครงการนี้ คณะผู้วิจัยได้ผลิตแอนติบอดีสายเดี่ยวของมนุษย์ (HuScFv) ที่จำเพาะต่อโปรตีนของไวรัสเด็งกี และทดสอบการจับและยับยั้งการทำงานของโปรตีน โดยมีวัตถุประสงค์ที่จะผลิตชีวโมเลกุล เพื่อใช้สำหรับรักษาการติดเชื้อไวรัสเด็งกี

คณะผู้วิจัยได้ผลิตแอนติบอดีสายเดี่ยวโดยการใช้รีคอมบิแนนท์โปรตีนของไวรัสเด็งกีชนิด rNS1, full-length envelope (rFL-E) และ domain III (rEDIII) เป็นแอนติเจนในการคัดเลือกแอนติบอดีสายเดี่ยวจาก Human antibody phage display library ซึ่งได้โคลนในแต่ละกลุ่มเป็นจำนวนมาก แต่ได้ทำการคัดเลือกจนเหลือโคลนที่จับได้ดีที่สุด สำหรับโปรตีน NS1 ได้จำนวน 2 โคลน (HuScFv11/HuScFv13) ซึ่งสามารถจับได้กับ rNS1 และ native NS1 และเมื่อนำไปทดสอบกับเซลล์ที่ติดเชื้อไวรัสเด็งกี พบว่าสามารถลดจำนวนอนุภาคไวรัสลงได้ แสดงว่าแอนติบอดีสายเดี่ยวที่ใช้ทดลอง อาจจะมีผลต่อการสร้างรูปร่างของอนุภาคไวรัสหรือต่อการปลดปล่อยไวรัสออกจากเซลล์ สำหรับการคัดเลือกโดยใช้โปรตีน rEDIII ได้จำนวน 1 โคลน (HuScFv15A) ซึ่งสามารถยับยั้งการติดเชื้อไวรัสเด็งกีของ Vero cells ในลักษณะผันแปรตามปริมาณของแอนติบอดี การศึกษา epitope mapping และ molecular docking ได้ผลว่ามีการจับของ HuScFv15A ต่อโครงสร้างที่ทำหน้าที่บริเวณ EDIII ของโปรตีน envelope ของไวรัสเด็งกี แม้จะยังจำเป็นต้องศึกษาเพิ่มเติมต่อไป แอนติบอดีสายเดี่ยวเหล่านี้ มีศักยภาพที่จะนำมาพัฒนาเพื่อให้เป็นชีวโมเลกุลสำหรับใช้ต้านไวรัสเด็งกีต่อไปได้

นอกจากนี้ คณะผู้วิจัยได้ใช้วิธี molecular docking เพื่อค้นหายาต้านไวรัสเด็งกีที่มีความปลอดภัย ซึ่งมุ่งในการหาเปปไทด์ (peptide) โดยได้คัดเลือกเปปไทด์ที่มีเป้าหมายในการจับที่ hydrophobic pocket บนโปรตีน envelope ของไวรัสเด็งกี มีบทบาทต่อการเปลี่ยนแปลงทางโครงสร้าง เมื่อมีการหลอมรวมระหว่างเยื่อหุ้มไวรัสกับเยื่อหุ้มเซลล์ เมื่อมีการติดเชื้อไวรัสเด็งกี นอกจากนี้ยังใช้ข้อมูลการจับกันของสารซึ่งมีรายงานการเกี่ยวกับการจับกับบริเวณ hydrophobic pocket มาประกอบการพิจารณาออกแบบเปปไทด์ด้วย ผลการทดลองปรากฏว่า ได-เปปไทด์ ชนิด EF สามารถยับยั้งไวรัสเด็งกี ซีโรทัยป์สอง (DENV2) ได้มีประสิทธิภาพสูงสุด โดยมีค่าครึ่งหนึ่งของความเข้มข้นในการยับยั้ง (IC50) เท่ากับ 96 μM การใช้เปป-ไทด์ EF ที่ความเข้มข้น 200 μM ทำให้มีการลดลงของจีโนมและโปรตีนอี (E) ภายในเซลล์เท่ากับร้อยละ 83 และร้อยละ 84 ตามลำดับ ในบรรดาไวรัสเด็งกี 4 ซีโรทัยป์ ปรากฏว่าชนิดซีโรทัยป์ 2 ให้ผลการยับยั้งดีที่สุด ผลการทดลองนี้เป็นการพิสูจน์ให้เห็นหลักการที่จะพัฒนาเปปไทด์ยับยั้งสำหรับเป็นยาต้านการติดเชื้อไวรัสเด็งกีโดยใช้คอมพิวเตอร์ในการช่วยออกแบบได้

คำสำคัญ: อณูพันธุศาสตร์; อณูชีววิทยา; โรคเบาหวาน; โรคนิ่วไต; โรคไตผิดปกติในการขับกรด; ไวรัสเด็งกี; พยาธิกำเนิด; แอนติบอดีสายเดี่ยวของมนุษย์; เปปไทด์ยับยั้ง

Contents

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Objectives:

Main Objectives:

(1) To identify disease-causing or disease-susceptibility genes involving in complex diseases (diabetes mellitus and kidney stone) in Thais.
(2) To elucidate molecular mechanisms of pathogeneses of these diseases (diabetes mellitus and kidney stone).
(3) To understand normal and abnormal trafficking mechanisms of membrane protein (distal renal tubular acidosis and kidney anion exchanger 1).
(4) To elucidate molecular pathogenesis of liver injury in infectious disease (dengue virus infection).
(5) To produce potential therapeutic molecules for treatment of infectious disease (dengue virus infection).
(6) To train graduate students and post-doctoral researchers in the area of molecular medicine.

Specific objectives for each project:

Objectives of Project I:

(1.1) To identify novel MODY genes by genome-wide linkage analysis in Thai MODY families.
(1.2) To identify and characterize the genes involved in T2D.
(1.3) To evaluate functional impact of novel genetic mutations and polymorphisms identified in Thai MODY and T2D patients.
(1.4) To evaluate effects of estrogen and testosterone on oxidative and ER stresses and apoptotic cell death in mouse pancreatic islets and β-cells cultured in high glucose medium.

Objectives of Project II:

(2.1) To identify disease-causing or susceptibility genes of kidney stone disease in northeastern Thai patients.
(2.2) To elucidate molecular mechanism of pathogenesis of kidney stone disease.
(2.3) To develop molecular genetic testing for disease-causing or susceptibility genes of kidney stone disease.

Objectives of Project III:

(3.1) To observe interaction between kAE1 and candidate trafficking-related proteins.
(3.2) To characterize roles of candidate trafficking-related proteins on kAE1 localization in non-polarized and polarized cultured cells.
(3.3) To examine effects of kAE1 trafficking-related proteins in kAE1 sorting in the secretory pathway from its biosynthesis site to cell surface and trafficking defects caused by dRTA-associated *SLC4A1* mutations.

Objectives of Project IV:

(4.1) To determine inflammatory cytokine and cell death responses induced by DENV infection in hepatic cell line.
(4.2) To determine inflammatory cytokine and cell death responses induced by DENV C and DENV NS5 in hepatic cell line.
(4.3) To determine molecular mechanisms how DENV C and DENV NS5 affect the inflammatory cytokine and cell death responses response in hepatic cell line.

Objectives of Project V:

(5.1) To produce human single chain antibody variable fragments (HuScFv) specific to dengue virus proteins.
(5.2) To test binding activity of HuScFv to their corresponding antigens.
(5.3) To test inhibitory activity of HuScFv to dengue virus infection/replication in cultured cells.

Introduction

Human genome, as same as genomes of all other species, is not static but subject to variation. This variation gives rise to phenotypic variability in the population important for adaptation of human species in changing environments and driving human evolution. However, human genomic variation can also cause human disease and illness. Many human diseases are caused exclusively by genetic abnormalities but more often they are complex disorders occurring from the interplay between human genetic make-up and environmental factors. Since human populations inhabit in differently changing environments, evolutionary process generates great genetic (allele and locus) heterogeneity for human disease by introducing different variations in each population to interact with differently changing environment. Thus, same human disease usually occurs from diverse causes and pathogenesis processes attributable to marked genetic heterogeneity in different populations.

The Human Genome Project (HGP) that announced its success in April 2003 has provided an explosive amount of information and new knowledge, which has been highly beneficial to human genetic and genomic research. In the past decade, the availability of a complete human genome sequence, coupled with significant technological advances, has revolutionized biomedical research to understand human health and disease. Using the human genome sequence and new technologies, we are now able to conduct biomedical research at a different level and pace. Application of genomic, proteomic, and bioinformatics technologies has now made it possible for human diseases to be studied on an unprecedented scale (1, 2). A whole spectrum of approaches to investigate human molecular biology in normal and disease conditions will provide a better understanding of normal body function and disease pathogenesis at the molecular level. This will potentially allow researchers and physicians to use this knowledge in the design of specific molecular tools for disease diagnosis, treatment, prognosis, and prevention – the field that is known as "Molecular Medicine" (3).

Recently, compelling evidence suggests that rare mutations with severe effect are responsible for a substantial portion of complex human disease and genetic heterogeneity is important at multiple levels of disease causation (4). These have been demonstrated by that, firstly, complex human diseases are substantially caused by collection of individually rare mutations; secondly, the same gene causing complex human disease may contain many (hundreds or thousands) different rare severe mutations in unrelated affected individuals; thirdly, the same mutation may cause different clinical manifestations (phenotypes) in different individuals; and fourthly, the same disorder may result from mutations in different genes in the same or related pathways. This extent of allelic, locus, and phenotypic heterogeneity has significant implication for gene discovery and development of molecular treatments for appropriate use in individual patients (4).

Based on this information, we propose that complex human diseases in Thais are caused by rare mutations with severe effect of the responsible genes that possess genetic heterogeneity. Since it is not possible to use information obtained from the studies in one population to explain subtle molecular pathogenesis of complex human diseases in another, it is necessary to directly investigate into these diseases in Thai patients. The availabilities of sequence information of human genome and powerful molecular biology techniques make it possible to conduct this investigation into the diseases that cause health and economic burdens to Thais. Our group has experiences for many years in the study of genetics and molecular biology of diabetes (5-12), kidney diseases (13-36), and dengue virus infection (37-45) in Thais. Currently, we are focusing our interest in the investigation of these diseases by applying advanced technologies in genetics, genomics, and molecular biology.

In this project, we propose to conduct the studies by taking advantage of currently developed genomic and molecular biology technologies to investigate into molecular pathogeneses of diabetes, kidney diseases, and dengue virus infection in Thais. These advanced technologies are not only powerful for investigation into biological processes at the molecular level but also highly potential for the development and testing of novel therapeutic molecules and drugs. In the present work, we have concurrently conducted five projects including:

Project I: Genetics/genomics and molecular biology of diabetes mellitus.
Project II: Genetics/genomics and molecular biology of kidney stone disease.
Project III: Molecular mechanism of anion exchanger 1 trafficking associated with distal renal tubular acidosis.
Project IV: Molecular pathogenesis of dengue virus infected liver cells.
Project V: Production of human single chain antibody variable fragments and peptide inhibitors specific to dengue virus proteins.

Project I: Genetics/Genomics and Molecular Biology of Diabetes Mellitus

Background/Rationale of the Problem for Research and Its Significance:

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia and the prolonged hyperglycemia results in complications including retinopathy, nephropathy, neuropathy, and cardiovascular disease. DM is classified into two main types: type 1 diabetes (T1D) and type 2 diabetes (T2D). The patients with T1D have an absolute insulin insufficiency due to immunological destruction of pancreatic β-cells that synthesize and secret insulin. T1D accounts for small proportion approximately 10% of all DM patients. T2D is a heterogeneous group of metabolic disorder, characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or combination of both. It is the most frequent form of diabetes accounting for 90% of all DM patients, affecting more than 170 million individuals worldwide with rapidly increasing in its incidence.

T2D is a genetically heterogeneous disease resulted from defects or variations of single or multiple genetic loci, in addition to the effects of environmental factors and lifestyle. Although genetic studies demonstrated that T2D in a majority of families is inherited as a non-Mendelian (multifactorial) pattern, a small proportion (~10%) of T2D was found to segregate in family as a Mendelian (monogenic) disorder. This monogenic T2D is classified as maturity onset diabetes of the young (MODY), which is characterized by young age at onset with autosomal dominant inheritance. It is now known that defects of at least 13 different genes result in MODY with the 6 well-characterized genes including *HNF-4α*, *GCK*, *HNF-1α*, *IPF-1*, *HNF-1β*, and *NeuroD11* but a number of MODY families have no mutations in these 13 genes, which are referred to as MODY-X.

Identification of genes causing MODY and that influencing individual susceptibility to T2D leads to a better understanding of pathophysiology of diabetes. Currently, two genetic approaches, linkage analysis and association study, are used for identification of disease and susceptibility genes. Linkage analysis is derived from genetic relationship between loci or phenotype and marker according to family-based studies; while association study is based on principle of linkage disequilibrium (LD), which is simply a statistical observation of the relationship between alleles and phenotypes in relation to population-based studies. Both linkage analysis and association study can be performed by genome-wide mapping or selection of candidate genes or chromosomal regions from their biologically function. A genome-wide linkage analysis will point out chromosome regions associated with disease. Fine mapping with another set of genetic markers is required for narrowing the regions of interest, which contains a set of plausible candidate genes. Association study will be subsequently used to find candidate genes.

Although there are multiple sources of genetic variation that occur among individuals, single nucleotide polymorphisms (SNPs) are the most common type of sequence variation and powerful markers in their abundance, stability and relative ease of genotyping. SNPs are currently selected as a marker for genome-wide linkage analysis and association studies. The improvements in technology have made it possible to conduct genome-wide mapping by genotyping of 500,000 SNPs or more in a single array, termed as microarray. Combining the information on genetic variations with new technological development in fields of bioinformatics, genomics and proteomics will provide a better chance to identify of novel candidate genes causing or influencing susceptibility to the disease.

Our research group, namely 'Siriraj Diabetes Mellitus Research Group (SiDMRG)', has been recruiting the patients with MODY and early-onset T2D as well as the patients with adult-onset T2D to investigate their genetic variations. The preliminary result showed that the six known MODY genes account for a small proportion of MODY and early-onset T2D patients, suggesting that MODY-X is more common in this group of Thai patients. New genes that cause MODY-X in Thai patients are being searched by candidate-gene approach and *PAX4* encoding PAX4 transcription factor that plays an important role in β-cell development has been reported by our group to cause MODY subtype 9 (MODY9) in Thai patients. It is also possible to apply the genome-wide linkage approach to identify novel candidate genes that cause MODY and early-onset T2D in Thai patients. Additionally, T2D-susceptibility genes in adults and the genes that involve in its complications are being investigated by the candidate-gene approach. The genetic mutations and polymorphism identified will be further examined into their biologically functional impact by molecular and cellular biology techniques. This molecular genetic information as well as molecular and cellular biology information, which are most likely to be different from what is described in other populations, is valuable for understanding molecular mechanisms underlying pathogeneses of MODY and T2D in Thai patients and would facilitate more appropriate measures for prevention, control, and treatment of diabetes in Thai.

Furthermore, prolonged hyperglycemia in the patients with T2D results in complications of macrovascular and microvascular diseases. Although glucose is a major source of energy to stimulate insulin secretion from pancreatic β-cells, it can also stimulate pancreatic β-cells to hypertrophy and hyperplasia. On the other hand, prolonged exposure of pancreatic β-cells to high glucose can result in impaired insulin synthesis and secretion or even cause pancreatic β-cell death – the event that is known as glucotoxicity. There are several mechanisms that cause glucotoxicity of pancreatic β-cells, one of which is oxidative stress because of low levels of antioxidant enzymes in the pancreatic β-cells. The increase of reactive oxygen species (ROS) may occur from hexosamine metabolism, sorbitol metabolism, protein kinase C (PKC) activation, and glyceraldehyde autooxidation.

It has been observed that decreased sex hormone levels are associated with the prevalence of diabetes. In male, declining free testosterone level was found to be associated with increasing diabetic incidence. In a similar manner, in menopause female, declining estrogen was found to be associated with elevated fasting plasma glucose. The mechanism of sex hormone affecting the increased prevalence of diabetes is still unknown. The result of our previous study showed that estrogen could increase insulin secretion in mouse islet after prolonged culture in a high glucose condition. We hypothesize that estrogen may exert the role in reduction of oxidative and/or ER stress in β-cells in prolonged exposure to a high glucose condition. We will therefore investigate this role of estrogen and its mechanism to reduce oxidative and/or ER stress in β-cells which are subject to prolonged culture in a high glucose condition. This information will be valuable for the development of method for prevention and treatment of diabetes in elderly people especially postmenopausal females.

Research Activities and Results in the Past Three Years

Recent genome-wide association studies (GWAS) in large cohorts from several ethnic groups have identified several genetic variations responsible for T2D susceptibility. These genes included, but not limited to, *TCF7L2*, *SLC30A8*, *HHEX*, *FTO*, *KCNQ1*, *CDKAL1*, *IGF2BP2* and *CDKN2A/2*, which encode proteins that most of them expressed in the pancreas and regulating pancreatic endocrine cell development and function. Associations of *TCF7L2* variant with T2D were replicated in several populations. In addition, several candidate genes that play crucial roles in regulating pancreatic β-cell development and function have been investigated. In this regard, we are the first group who identified the mutations and single nucleotide polymorphisms (SNPs) of *PAX4* caused MODY9 and T2D, respectively. Our group aim to examine whether genetic variations of *TCF7L2*, *CAPN10*, *AdipoQ*, and *PAX4* genes are associated with T2D in Thais. Most of genetic variations analyzed in this study are single nucleotide polymorphisms (SNPs), except for *CAPN10* by which copy number variation (CNV) are explored. In addition to case-control genetic association studies, we also examined the functional defects on either transcriptional regulation on target-gene promoters or protein multimerization.

For *TCF7L2* variations, 5 SNPs including rs7896340, rs7901695, rs7903146, rs12255372 and rs11196205 of *TCF7L2* were genotyped in Thai patients with T2D (n=202) and ethnically matched control subjects (n=205) by high resolution melting analysis using a real-time PCR machine. The genotypes of *TCF7L2* SNPs rs7896340 and rs11196205 were found to be associated with T2D in Thai patients in dominant inheritance model with p-values of 0.009 and 0.013, respectively. The odds ratios (ORs) calculated from genotype frequencies of minor and major alleles of the patients and control groups were 2.34 (95% CI 1.22-4.47) for rs7896340 and 2.33 (95% CI 1.18-4.26) for rs11196205. The haplotypes composed of GG minor alleles of these two SNPs were also significantly associated with T2D with ORs 1.89 [95% CI 1.12-3.19, p=0.018 (global p=0.013)]. Moreover, the patients who carried minor alleles of these two SNPS had earlier age-onset of diabetes (AA=50.32±10.78 years, AG+GG=45.51±10.52 years, p=0.012). These data suggested that *TCF7L2* variations are associated with T2D and earlier age-onset of the disease in Thai patients (46).

Genetic case-control association studies in Thais revealed that genotype distribution of *CAPN10* Indel19 was deviated from Hardy-Weinberg equation (HWE) after correction of genotyping error. These data indicated that there might have copy number variations (CNVs) within *CAPN10* Indel19 region. We conducted the semi-quantitative denaturating high performance liquid chromatography (DHPLC) method to detect CNVs. We successfully detected the CNVs within this region by DHPLC. After correction of genotype calling based on the status of identified CNVs, *CAPN10* Indel19 genotypes were well-fitted for HWE (p>0.05). However, we did not find association between CNV genotypes and T2D development. After excluding identified CNVs from the analysis, *CAPN10* Indel19 was associated with T2D. The information obtained from our study would be helpful for genotyping accuracies of SNPs residing in the CNVs region (47).

ADIPOQ, encoding adiponectin, is a candidate gene for T2D identified by genome-wide linkage analyses with supporting evidences showing the protein function in sensitizing insulin actions. In an endeavor to characterize candidate genes causing T2D in Thai patients, we identified 10 novel *ADIPOQ* variations, several of which were non-synonymous variations identified only in the patients. To examine the impact of these non-synonymous variations on adiponectin structure and biochemical characteristics, we conducted structural analysis of the wild-type and variant proteins by *in silico* modeling and further characterized biochemical properties of the variants with predicted structural abnormalities from the modeling by molecular and biochemical studies. The recombinant plasmids containing wild-type and variant *ADIPOQ* cDNAs derived from the variations

identified by our study (R55H, R112H, and R131H) and previous works (G90S and R112C) were constructed and transiently expressed and co-expressed in cultured HEK293T cells to investigate their oligomerization, interaction, and secretion. We found that the novel R55H variant impaired protein multimerization but it did not exert the effect over the co-expressed wild-type protein while novel R131H variant impaired protein secretion and also affect the co-expressed wild-type protein in a dominant negative fashion. The R131H variant could traffic from endoplasmic reticulum to Golgi, trans-Golgi network, and early endosome but could not be secreted. The R131H variant was likely to be degraded through the lysosomal system and inhibition of its degradation rescued the variant protein from secretion defect. We have shown the possibility of using *in silico* modeling for predicting the effect of amino acid substitution on adiponectin oligomerization. This is also the first report that demonstrates a dominant negative effect of the R131H variant on protein secretion and the possibility of using protein degradation inhibitors as therapeutic agents in the patients carrying adiponectin variants with secretion defect (48).

PAX4 R192H polymorphism was reported by our group to be associated with MODY and early onset-age of T2D. The transcriptional repression activity of *PAX4* R192H polymorphism was examined by luciferase reporter assay. Wild-type PAX4 and PAX4 R192H, which were found to be equally expressed *in vitro* and transfection systems, were present in the nuclear compartment. Transcriptional repressor activities of PAX4 R192H on human *insulin* and *glucagon* promoters were reduced when they were compared with those of wild-type PAX4. These results suggested that *PAX4* R192H polymorphism generated a protein with defect in transcriptional repressor activities on its target genes, which may lead to β-cell dysfunction associated with MODY and early onset-age of T2D as reported in our previous study (49). In addition, we found a novel *PAX4* mutation, IVS7-1G>A, in a Thai family with MODY9, with diabetic retinopathy, nephropathy and premature renal failure, suggesting the severity of this *PAX4* mutation. Since this *PAX4* IVS7-1G>A mutation disrupts the acceptor splice site in intron 7, we thus examined mRNA splicing of this *PAX4* mutation compared to that of *PAX4* wild-type by the minigene assay. The result showed that, when this acceptor splice site was disrupted, a nearly cryptic splice within exon 8 was used, causing a deletion of three nucleotides at the positions 748 to 750 (c.748_750del CAG), which resulted in a deletion of glutamine at the position 250 (p.Q250del). This amino acid is located nearby the repressor domain at the C-terminus of PAX4 protein and highly conserved among mammalian species. We examined the repressor function of PAX4 Q250del on human *insulin* and *glucagon* promoters in 832/13 derived INS-1 and αTC1.9 cells, respectively. The mutant PAX4 protein had a significantly decreased repressor functions on these two promoters compared to that of the wild-type PAX4. Western blot analysis showed similar levels of wild-type and Q250del protein expression and immunofluorescence assay revealed normal translocation of the wild-type and mutant proteins into the nucleus. β-cell apoptosis was increased in INS-1 overexpressed PAX4 Q250del culturing under glucotoxic stress condition. In conclusion, we reported a novel *PAX4* IVS7-1G>A mutation, causing aberrant mRNA splicing that generated an inflame deletion of PAX4 protein (Q250del). This mutant PAX4 had impaired repressor functions on target-gene promoters and increased susceptibility to cell apoptosis upon high glucose exposure, which may lead to β-cell dysfunction, β-cell death and contribute to the pathogenesis of diabetes in this MODY9 family (manuscript to be submitted for publication).

In addition to the genetic analysis, we also investigated the mechanism of sex hormone-mediated against glucotoxicity of β-cells for providing an alternative strategy for treatment of diabetes. There have been evidences demonstrating that estrogen can improve glucose homeostasis not only in diabetic rodents but also in humans, and testosterone can protect pancreatic β-cells from glucotoxicity. However, the molecular mechanism by

which estrogen and testosterone prevent pancreatic β-cell death remains unclear. To investigate this issue, INS-1 cells, a rat insulinoma cell line, were cultured in medium with either 11.1 mM or 40 mM glucose in the presence or the absence of estrogen or testosterone. Estrogen significantly reduced apoptotic β-cell death by decreasing nitrogen-induced oxidative stress and the expression of the ER stress markers GRP 78, ATF6, P-PERK, PERK, uXBP1, sXBP1, and CHOP in INS-1 cells after prolonged culture in medium with 40 mM glucose. In contrast, estrogen increased the expression of survival proteins, including sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA-2), Bcl-2, and P-p38, in INS-1 cells after prolonged culture in medium with 40 mM glucose. The cytoprotective effect of estrogen was attenuated by addition of the estrogen receptor (ERα and ERβ) antagonist ICI 182,780 and the estrogen membrane receptor inhibitor G15. We showed that estrogen decreases not only oxidative stress but also ER stress to protect against 40 mM glucose-induced pancreatic β-cell death (50).

For testosterone, any effect on apoptosis and viability of INS-1 cells cultured in basal glucose medium were not changed, but it could reduce apoptosis and increase viability of INS-1 cells cultured in high-glucose medium. The protective effect of testosterone is diminished by androgen receptor inhibitor. Testosterone could significantly reduce nitrotyrosine levels, mRNA, and protein levels of ER stress markers and sensors those were induced, when INS-1 cells were cultured in high-glucose medium. It could also significantly increase the survival proteins, sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA-2) and Bcl2, in INS-1 cells cultured in the same conditions. Similarly, it could reduce ER stress markers and increase SERCA protein levels in male mouse pancreatic islets cultured in high glucose medium. Testosterone can protect against male pancreatic β-cell apoptosis from glucotoxicity via reduction of both oxidative stress and ER stresses (51).

Project II: Genetics/Genomics and Molecular Biology of Kidney Stone

Background/Rationale of the Problem for Research and Its Significance:

Kidney stones are an important public health problem in the Northeastern (NE) population of Thailand. Its prevalence is 5-10% with several thousands of new cases hospitalized for treatment each year. The etiology and pathogenesis of kidney stone are still obscure. However, the kidney stone disease in this population seems to be unique from what has been reported in other ethnic groups because it is not associated with the conditions of increased urinary stone promoters, such as hypercalciuria, hyperoxaluria, and hyperuricosuria. Our group has recently reported an initial evidence suggesting a genetic contribution to kidney stone in the NE Thai population because it was found to have familial aggregation with a high relative risk (λ_R = 3.18) among members of the affected families. Additionally, from the data of our study in about 200 families with kidney stone, the inheritance of this disease within the families is clear in many families.

A number of genetic abnormalities resulting in kidney stone diseases have been identified and reported in animal species and human populations. However, these abnormalities are unlikely to cause the kidney stone disease in the northeastern Thai patients because the phenotypes associated with these genetic abnormalities, for examples, hypercalciuria, hyperoxaluria, and hyperuricosuria, are not present in most of the northeastern Thai patients with kidney stone. In the past several years, our group have conducted clinical studies and collected genetic data as well as clinical (blood, urine, and stone) samples from patients and their family members at two hospitals in northeastern Thailand, Khon Kaen and Sappasitthiprasong (Ubon Ratchathani) Hospitals. The uniqueness and the presence of clinical and genetic data as well as the DNA sample collections make us in an advantageous position to conduct genetics and molecular biology of kidney stone in the northeastern Thai population.

Modern genomic technologies provide powerful tools and methods for identification of genes causing or involving in genetic diseases. One of these tools is a high-throughput single nucleotide polymorphism (SNP) genotyping platform or DNA microarray. It is a mapping tool designed to identify regions of the genome that are linked to or associated with, a particular trait or phenotype. To investigate into the role of genetic factor in pathogenesis of kidney stone in the northeastern Thai population, we have employed multiple genetic or genomic approaches, such as genome-wide linkage and association studies using DNA microarray and candidate-gene association study, to identify disease or susceptibility genes.

Genome-wide linkage analysis was performed in four extended families having several members with kidney stone disease. Fifty-three subjects were analyzed by a genome-wide scan using Affymetrix GeneChip® Mapping 10K Xba 142 2.0 Array. Two-point, multipoint parametric and non-parametric analyses were conducted with the software package, easyLINKAGE. Significant linkages (LOD>3) on 3p22.3 and 7q31.1 together with positive results of suggestive linkages (LOD>2) on 11q24.1, 11q24-25, 12q23.1, 17q25.1, and18q21-23 were observed. Nine candidate genes (*GPD1L, CAV1*, *CAV2*, *SLC9A3R1, BLID*, *MIRN125B1*, *MIRNLET7A2*, *MIRN100* and *KCNJ1*) were selected from these linkage loci to analyze by DNA sequencing but no genetic variation associated with the disease was identified. Fine mapping and identification of candidate genes within these regions are under further investigation. In parallel, genome-wide association study was initially performed in fifty-five subjects each of the patient and control groups by using DNA microarray (Genome-Wide Human SNP Array 6.0, Affymetrix). Data of SNP typing from both groups were analyzed for their association with the disease using GenABEL (an R package). After correction for multiple-SNPs test with empirical GW significance, 71 SNPs showed significant differences in frequencies between the case and control groups ($P< 0.05$). These SNPs were located pervasively on the genome except chromosomes 16, 17, 19, and Y. However, the number of samples analyzed might not be adequate and a larger sample size should be studied to increase power of the test, which is being carried out as a new project.

In this project, we continue and extend the study to identify the genes causing or involving in kidney stone disease in the northeastern Thai patients and to elucidate its molecular pathogenesis by genetic/genomic and molecular biology techniques. The initial data obtained from our studies by genome-wide linkage analysis and genome-wide association study using DNA microarray will be taken for selection of candidate genes for further examination by sequencing, mutation and segregation analysis, and assessment in a larger sample size. If the disease causing or susceptibility gene is identified, the encoded protein will be further investigated into its function and association with molecular pathogenesis of the disease. The knowledge and technology obtained and developed from this project will lead to a better understanding and improvement of prevention, control, and management of kidney stone in the northeastern Thai patients.

Research Activities and Results in the Past Three Years

We firstly conducted a candidate gene association study by genotyping 67 SNPs within 8 genes including *TFF1, S100A8, S100A9, S100A12, AMBP, SPP1, UMOD*, and *F2*, encoding urinary stone-inhibitor proteins: trefoil factor 1, calgranulin (A, B, and C), bikunin, osteopontin, Tamm-Horsfall protein, and urinary prothrombin fragment 1, respectively, in 112 subjects each of the patient and control groups. Significant differences between the case and control groups of allele and genotype frequencies of 8 SNPs in *F2* were found while those of the remaining 7 genes were not. This finding indicated that the association between *F2* and the risk of kidney stone disease in Northeastern Thai patients. Then, we sequenced entire coding regions of *F2* to identify specific variation associated with kidney stone disease. We have now found one exonic non-synonymous single

nucleotide polymorphism (nsSNP; rs5896); c.494 C>T) which located in exon 6 results in a substitution of threonine (T) by methionine (M) at the position 165 (T165M). The nsSNP rs5896 was subsequently genotyped in 209 patients and 216 control subjects. Genotypic and allelic frequencies of this nsSNP were analyzed for their association with kidney stone disease. The frequency of CC genotype and the frequency of C allele of rs5896 were significantly lower in the patient group than that in the control group. The effect of amino-acid change on UPTF1 structure was also examined by homologous modeling and in silico mutagenesis. T165 is conserved and T165M substitution will affect hydrogen bond formation with E180. Moreover, we found that minor allele and homozygous genotype frequencies of 8 of 10 SNPs distributed within the *F2* gene were significantly higher in the control group than in the patient group. Two *F2* haplotypes were found to be dually associated with kidney stone risk, one (TGCCGCCGCG) with increased disease risk (P = 0.0013, OR 1.612, 95% CI 1.203-2.160) and the other (CGTTCCGCTA) with decreased disease risk (P = 0.0007, OR 0.464, 95% CI 0.296-0.727) (52-53). However, these 2 haplotypes and the significant differences of CC genotype and C allele frequencies of rsSNP5896 were maintained only in the female group. Our results indicate that prothrombin variant (T165M) is associated with kidney stone risk in the Northeastern Thai female patients. Ongoing of this project, we are using exome sequencing to identify other inherited causes of kidney stone disease and characterize the function by using molecular biology and cellular techniques.

Furthermore, a whole genome SNP genotyping by DNA microarray were initially conducted in another 101 patients and 105 control subjects. A set of 103 candidate genes reported to be involved in KSD, gathered from public databases and candidate gene association study databases, were evaluated for their variations associated with KSD. Altogether 82 SNPs distributed within 22 candidate gene regions showed significant differences in SNP allele frequencies between the patient and control groups ($P < 0.05$). Of these, 4 genes including *BGLAP*, *AHSG*, *CD44*, and *HAO1*, encoding osteocalcin, fetuin-, CD44-molecule and glycolate oxidase 1, respectively, were further assessed for their association with the disease because they carried high proportion of SNPs with statistical differences of allele frequencies between the patient and control groups within the gene. The total of 26 SNPs showed significant differences of allele frequencies between the patient and control groups and haplotypes associated with disease risk were identified. The SNP rs759330 located 144 bp downstream of *BGLAP* where it is a predicted microRNA binding site at 3'UTR of *PAQR6* – a gene encoding progestin and adipoQ receptor family member VI, was genotyped in 216 patients and 216 control subjects and found to have significant differences in its genotype and allele frequencies (P = 0.0007 and 0.0001, respectively). Our results suggest that these candidate genes are associated with KSD and *PAQR6* comes into our view as the most potent candidate since associated SNP rs759330 may affect mRNA expression level (manuscript under review).

We selected a large family with KSD (UBRS082) to perform a genome-wide linkage and exome sequencing. There are 7 and 10 family-members who were affected and unaffected, respectively, and KSD phenotype was inherited as autosomal dominant model. Chromosomal regions with high logarithm of odd scores (LOD >2.80) were initially identified by genome-wide linkage and genetic variations in these regions were examined by exome sequencing. Two novel variations (p.N909K and p.K1809R) of *SCN10A* were co-segregated with KSD in one affected family without presence in normal control subjects (n=180). As these two variations were co-inherited in the same allele, they might have combined effects in causing KSD. An additional variation (p.V1149M) of *SCN10A* was identified in another affected family. Nav1.8α subunit mRNA and protein are expressed in human kidney tissues. All these findings provide strong evidences supporting that the mutations of SCN10A cause familial KSD (manuscript submitted for publication).

Project III: Molecular Mechanism of Anion Exchanger 1 Trafficking Associated with Distal Renal Tubular Acidosis

Background/Rationale of the Problem for Research and Its Significance:

Kidney anion exchanger 1 (kAE1) is the basolateral Cl^-/HCO_3^- exchanger of the acid-secreting type A intercalated cells of the kidney involved in maintaining acid-base homeostasis in the human body. Several mutations in the *SLC4A1* gene encoding kAE1 have been found to be associated with both autosomal dominant and autosomal recessive distal renal tubular acidosis (dRTA), characterized by an inability of the kidney to secrete H^+ into urine resulting in systemic metabolic acidosis often accompanied by several clinical manifestations including muscle weakness, growth retardation, metabolic bone disease, nephrocalcinosis, nephrolithiasis, chronic pyelonephritis, and renal failure. The *SLC4A1* mutations associated with dRTA do not cause defect of the protein in Cl^-/HCO_3^- exchange function but result in impaired trafficking or mistargeting of the mutant kAE1 proteins. Characterization of dRTA-associated *SLC4A1* mutations using transfected cell systems demonstrated that *SLC4A1* mutations affect the intracellular trafficking of kAE1 protein to the basolateral surface, either accumulating within intracellular compartment or mistargeting kAE1 protein to inappropriate destinations. However, it has yet been unknown how the protein trafficking fails or why mistargeting of kAE1 protein occurs.

Protein transport along the secretory pathway is a tightly regulated process and requires specific protein-protein interactions and recognitions between cargo molecules and trafficking machinery to achieve correct targeting of cargo proteins to their destinations. Mutations that disrupt such interactions or cause protein misfolding often impair the transport process. To understand the pathogenesis of dRTA caused by *SLC4A1* mutations, it is necessary to investigate the transport and targeting process of kAE1 protein from its biosynthesis site to the cell surface in both normal and abnormal conditions.

Research Activities and Results in the Past Three Years

Distal renal tubular acidosis (dRTA) caused by mutations of the *SLC4A1* gene encoding the erythroid and kidney isoforms of anion exchanger 1 (AE1 or band 3) has a high prevalence in some tropical countries, particularly Thailand, Malaysia, the Philippines and Papua New Guinea (PNG). Here, the disease is almost invariably recessive and can result from either homozygous or compound heterozygous *SLC4A1* mutations. For genetic study, we have collected and reviewed our own and published data on tropical dRTA to provide a comprehensive series of clinical and epidemiological studies in 78 patients. Eight responsible *SLC4A1* mutations have been described so far, four of them affecting multiple unrelated families. With the exception of the mutation causing South-East Asian ovalocytosis (SAO), none of these mutations has been reported outside the tropics, where dRTA caused by *SLC4A1* mutations is much rarer and almost always dominant, resulting from mutations that are quite different from those found in the tropics. *SLC4A1* mutations, including those causing dRTA, may cause morphological red cell changes, often with excess haemolysis. In dRTA, these red cell changes are usually clinically recessive and not present in heterozygotes. The high tropical prevalence of dRTA caused by *SLC4A1* mutations is currently unexplained. A hypothesis suggesting that changes in red cell metabolism caused by these mutations might protect against malaria is put forward to explain the phenomenon, and a possible mechanism for this effect is proposed (54).

For kAE1 functional study, despite much evidence suggesting that the C-terminal portion of kAE1 is involved in basolateral membrane trafficking, very little information is known about proteins that physically interact with the C-terminal tail of kAE1. Our group is interested in identifying the protein that interacts with kAE1 and plays a role in its basolateral trafficking. To investigate the trafficking process of kAE1 and why mutant

kAE1 fail to transport, our group has identified interacting proteins of kAE1 in human kidney using yeast two hybrid (Y2H) screening. Proteins specifically interacting with kAE1 (either N- or C-terminus) have been screened and isolated from a human kidney cDNA library. The partial sequences of prey-cDNAs obtained from the screening and sequencing were analyzed with homology BLAST search for full-length cDNA and proteins in the NCBI GenBank and EMBL databases. The specific interactions between kAE1 and some of kAE1-binding proteins (kAE1-BPs), integrin-linked kinase (ILK) and adaptor-related protein complex 1 mu1A (AP-1 mu1A), have been confirmed by co-immunoprecipitation, affinity co-purification, and co-immunofluorescence staining and further studies of ILK suggest its potential roles in the cell surface transport and/or membrane anchoring of kAE1 at the basolateral membrane. The interacting site for AP-1 mu1A on Ct-kAE1 was found to be Y904DEV907, a subset of YXXØ motif. Interestingly, suppression of endogenous AP-1 mu1A in HEK 293T by small interfering RNA (siRNA) decreased membrane localization of kAE1 and increased its intracellular accumulation, suggesting for the first time that AP-1 mu1A is involved in the kAE1 trafficking of kidney α-intercalated cells (55). Moreover, the interaction between kAE1 and mu-1A and B in vitro by reciprocal coimmunoprecipitation in epithelial cells and in vivo by coimmunoprecipitation from mouse kidney extract. When endogenous mu-1A (and to a lesser extent mu-1B) was reduced, kAE1 protein was unable to traffic to the plasma membrane and was rapidly degraded via a lysosomal pathway. Expression of either small interfering RNA-resistant mu-1A or mu-1B stabilized kAE1 in these cells. We also show that newly synthesized kAE1 does not traffic through recycling endosomes to the plasma membrane, suggesting that AP-1B, located in recycling endosomes, is not primarily involved in trafficking of newly synthesized kAE1 when AP-1A is present in the cells. Our data demonstrate that AP-1A regulates processing of the basolateral, polytopic membrane protein kAE1 to the cell surface and that both AP-1A and B adaptor complexes are required for normal kAE1 trafficking (56).

In the sorting process, kAE1 interacts with AP-1 mu1A, a subunit of AP-1A adaptor complex. However, it is not known whether kAE1 interacts with motor proteins in its trafficking process to the plasma membrane or not. We have discovered that kAE1 interacts with kinesin family member 3B (KIF3B) in kidney cells and a dileucine motif at the carboxyl terminus of kAE1 contributes to this interaction. We have also demonstrated that kAE1 co-localizes with KIF3B in human kidney tissues and the suppression of endogenous KIF3B in HEK293T cells by small interfering RNA (siRNA) decreases membrane localization of kAE1 but increases its intracellular accumulation. All results suggest that KIF3B is involved in the trafficking of kAE1 to the plasma membrane of human kidney a-intercalated cells (57).

However, it is not known how the intracellular sorting and trafficking of kAE1 from trans-Golgi network (TGN) to the basolateral membrane occur. Here, we studied the role of basolateral-related sorting proteins, including mu1 subunit of adaptor protein (AP) complexes, clathrin, and protein kinase D, on kAE1 trafficking in polarized and non-polarized kidney cells. By using RNA interference, co-immunoprecipitation, yellow fluorescent protein-based protein fragment complementation assay, and immunofluorescence staining, we demonstrated that AP-1 mu1A, AP-3 mu1, AP-4 mu1 and clathrin (but not AP-1 mu1B, PKD1 or PKD2) play crucial roles in intracellular sorting and trafficking of kAE1. We also demonstrated co-localization of kAE1 and basolateral-related sorting proteins in human kidney tissues by double immunofluorescence staining. These findings indicate that AP-1 mu1A, AP-3 mu1, AP-4 mu1, and clathrin are required for kAE1 sorting and trafficking from TGN to the basolateral membrane of acid-secreting alpha-intercalated cells (58).

Project IV: Molecular Pathogenesis of Dengue Virus Infected Liver Cells

Background/Rationale of the Problem for Research and Its Significance:

Dengue hemorrhagic fever (DHF) is the major health problem worldwide, especially in tropical and sub-tropical areas. More than 2,500 million people in more than 100 countries are in the endemic areas with the incidence of infection approximately 50 million cases annually. Most patients are at school ages and also adults. In Thailand, first dengue case was reported in year 1950 with the first epidemic in Bangkok in year 1958. Since then, DHF is observed as the seasonal outbreak annually especially in the community areas. Dengue virus infection is transmitted to human via the biting of female *Aedes* mosquitoes harboring dengue virus in their salivary gland; thus, the transmission of the disease vector indicates the distribution of DHF. The clinical manifestations of infected individuals vary from mild febrile illness (dengue fever, DF), to the more severe form of disease characterized by bleeding in internal organs (DHF), and the fatal form when patient going into shock (dengue shock syndrome, DSS). The severe form of disease is usually found in patient with the secondary infection with the dengue virus in heterologous subtype from previous infection. The more severe clinical features are postulated to be due to the effect of virus-specific antibodies at sub-neutralizing level that could enhance the binding of virus to host cell antibody (Fc) receptor and facilitate viral entry in the following infection. Moreover, those antibodies with effecter sites (Fc) affect signaling pathway of the target cells and also stimulate the formation of complement attach complex resulting host cell lysis.

DENV belongs to the *Flaviviridae* family and its particle contains a single positive-stranded RNA genome, encoding a single precursor polypeptide. Host and viral proteases cleave this polypeptide into three structural proteins including capsid (C), membrane (M), and envelope (E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5), respectively. Clinical symptoms range from a predominantly febrile disease, dengue fever (DF), to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which usually occurs in cases with subsequent infection with a different serotype of DENV. The patients with DHF generally present with hemorrhagic tendencies, plasma leakage, thrombocytopenia, and hemoconcentration.

Liver cell injury is commonly observed in patients with DENV infections. Elevation of aminotransferases, reactive hepatitis and fulminant hepatic failure are frequently noticed in the patients with DHF/DSS. The cause of hepatocyte injury during DENV infection, which may lead to fulminant hepatic failure, remains unclear. We postulated that $CD4^+$ cytotoxic T cells may mediate liver damage through stimulation of inflammatory cytokines and cell death. In this project, we will determine inflammatory cytokine and cell death responses induced by DENV infection in cultured hepatic cells. Furthermore, the roles and mechanisms of DENV C and DENV NS5 proteins in the induction of inflammatory cytokine and cell death responses will be investigated in the cultured hepatic cells. The results of these studies will provide insight into molecular pathogenesis of DENV infection causing liver cell injury and will facilitate the development of new therapeutic modalities for DENV infection.

Research Activities and Results in the Past Three Years

Patients with DENV infection exhibit evidence of hepatocyte injury. However, the mechanisms of hepatocyte injury are unclear. Therefore we examined the expression of cell death genes during DENV-infection of HepG2 cells using real-time PCR arrays. The expression changes were consistent with activation of apoptosis and autophagy. Expression of the up-regulated genes, including *RIPK2*, *HRK*, *TGF*-β, *PERK*, and *LC3B*, was confirmed by quantitative real-time PCR. RIPK2 belongs to the receptor-interacting protein family of serine/threonine protein kinases, which is a crucial mediator of multiple stress responses that

leads to the activation of caspase, NF-κB and MAP kinases including JNK and p38. RIPK2 activity is inhibited by the p38 MAPK pathway inhibitor SB203580. The effect of SB203580 on RIPK2 expression and DENV-induced apoptosis was tested in DENV-infected HepG2 cells. The inhibition of RIPK2 expression by SB203580 significantly reduced apoptosis. SB203580 also significantly reduced DENV capsid protein (DENVC)-mediated apoptosis. Suppression of endogenous RIPK2 in DENV-infected HepG2 cells by small interfering RNA (siRNA) significantly decreased apoptosis suggesting for the first time that RIPK2 plays a role in DENV-mediated apoptosis (59).

From real-time PCR arrays, we also found *cathepsin* genes that have been shown to be up-regulated in DENV-infected HepG2 cells. Cathepsins, which are cysteine proteases inside the lysosome, were previously reported to be up-regulated in patients with DHF. However, their functions during DENV infection have not been thoroughly investigated. We show for the first time that DENV induces lysosomal membrane permeabilization. The resulting cytosolic cathepsin B and S contributed to apoptosis via caspase activation. The activity of caspase 3 was significantly reduced in DENV-infected HepG2 cells treated with cathepsin B or S inhibitors. Treatment with cathepsin B inhibitor also reduced the activity of caspase 9, suggesting that cathepsin B activates both caspase-9 and caspase-3. Reduced cathepsin B expression, effected by RNA interference, mimicked pharmacological inhibition of the enzyme and confirmed the contribution of cathepsin B to apoptotic events induced by DENV in HepG2 cells (60).

Furthermore, we compared the expression of cytokine genes between mock-infected and DENV-infected HepG2 cells using a real-time PCR array and revealed several up-regulated chemokines and cytokines, including *CXCL10* and *TNF*-α. Increased levels of cytokines - the so-called 'cytokine storm', contribute to the pathogenesis of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In this study, Compound A (CpdA), a plant-derived phenyl aziridine precursor containing anti-inflammatory action and acting as a dissociated nonsteroidal glucocorticoid receptor modulator, was selected as a candidate agent to modulate secretion of DENV-induced cytokines. CpdA is not a glucocorticoid but has an anti-inflammatory effect with no metabolic side effects as steroidal ligands. CpdA significantly reduced DENV-induced CXCL10 and TNF-α secretion and decreased leukocyte migration indicating for the first time the therapeutic potential of CpdA in decreasing massive immune activation during DENV infection (61).

We have previously described the DENV C are important for its nuclear localization, interaction with death-domain-associate (DAXX) protein and induction of apoptosis. A double substitution mutation in DENV C (R85A/K86A) abrogates DAXX interaction, nuclear localization and apoptosis. Therefore, we compared the expression of cell death genes between HepG2 cells expressing DENV C and DENV C (R85A/K86A) using a real-time PCR array. Expression of CD137, which is a member of the tumor necrosis factor receptor family, increased significantly in HepG2 cells expressing DENV C compared to HepG2 cells expressing DENV C (R85A/K86A). In addition, CD137-mediated apoptotic activity in HepG2 cells expressing DENV C was significantly increased by anti-CD137 antibody compared to that of HepG2 cells expressing DENV C (R85A/K86A) (62). In DENV-infected HepG2 cells, *CD137* mRNA and CD137 positive cells significantly increased and CD137-mediated apoptotic activity was increased by anti-CD137 antibody. CD137 recruits TNF receptor associated factor 2 (TRAF2) and activates apoptosis signal regulating kinase 1 (ASK1), resulting in activation of cJun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). p38 MAPK participates in both apoptosis-related signaling and pro- inflammatory cytokine production. The role of p38 MAPK in DENV-infected HepG2 cells was examined using RNA interference. The results showed that DENV infection activated p38 MAPK and induced apoptosis. The p38 MAPK activation and TNF-α production were controlled by p38 MAPK and CD137 signaling in

DENV-infected HepG2 cells as activated p38 MAPK, TNF-α and apoptosis were significantly decreased in p38 MAPK and CD137 depleted DENV-infected HepG2 cells. Addition of exogenous TNF-α to p38 MAPK depleted DENV-infected HepG2 cells restored DENV-induced apoptosis in HepG2 cells. DENV induces CD137 signaling to enhance apoptosis by increasing TNF-α production via activation of p38 MAPK (63).

However, the *in vivo* role of ERK1/2, a member of the MAPK family, and the question whether its activation can facilitate cell survival or cell death, has not been thoroughly investigated. Therefore, the role of ERK1/2 in a mouse model of DENV infection was examined. Our results show that DENV induces phosphorylation of ERK1/2 and increases apoptosis. Inhibition of phosphorylated ERK1/2 by the selective ERK1/2 inhibitor, FR180204, limits hepatocyte apoptosis and reduces DENV-induced liver injury. Clinical parameters, including leucopenia, thrombocytopenia, transaminases and histology, show improvements after FR180204 treatment. The expression of cell death genes was further identified using real-time PCR array and Western blot analysis. Caspase-3 was significantly decreased in FR180204 treated DENV-infected mice compared to the levels of untreated DENV-infected mice suggesting the role of ERK1/2 signaling in immune-mediated liver injury during DENV infection (64).

DAXX also was identified to interact with DENV nonstructural protein 5 (NS5) using yeast two-hybrid assay. The *in vivo* relevance of this interaction was suggested by co-immunoprecipitation and nuclear co-localization of these two proteins in HEK293 cells expressing DENV NS5. HEK293 cells expressing DENV NS5-K/A, which were mutated at the nuclear localization sequences (NLS), were created to assess its functional roles in nuclear translocation, DAXX interaction, and cytokine production. In the absence of NLS, DENV NS5 could neither translocate into the nucleus nor interact with DAXX to increase the DHF-associated cytokine, RANTES (CCL5) production. This work demonstrates the interaction between DENV NS5 and DAXX and the role of the interaction on the modulation of RANTES production (65).

Project V: Production of Human Single Chain Antibody Variable Fragments and Peptide Inhibitors Specific to Dengue Virus Proteins

Background/Rationale of the Problem for Research and Its Significance:

Dengue virus (DENV) infection is one of the most important mosquito-borne viral diseases, affecting many million people worldwide. The causative agents of the disease, dengue viruses, are in family *Flaviviridae* which are serologically categorized into 4 subtypes. The virus contains the positive-sense single stranded RNA genome encoding 10 proteins i.e. 3 structural (E, prM, and C) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. Among them, only E, prM, C, and NS1 could be recognized outside the infected cells. The structural proteins play major roles in the initial steps of infection which starts from virus binding, entry, membrane fusion, and genome release. In the late phase of infection, NS1 protein will be tremendously produced from the infected cells and can be detected in both secretory form in serum and membrane-associated form on infected cells. Secretory NS1 in serum can induce the formation of complement complex on vascular endothelial cells resulting in the plasma leakage.

On the other hand, host immune responses to infection also generate many pro- and anti-inflammatory mediators for immune regulation during the course of illness e.g. IL-6, IL-8, IL-10, IL-1β, TNF-α, MIF. These immunomodulating agents themselves promote the pathogenesis in the infected patient as it is termed "cytokine storm" in viral infection. Many studies suggested the correlation of elevated serum cytokine level with the disease severity and mortality. It was evidenced that the survival rate of infected animals was increased upon depletion of cytokines.

Nowadays, a vaccine and anti-viral agent with protective efficacy against dengue infection have not been available and the disease management is only for supportiveness. We propose that blocking or inhibiting of the functions of viral proteins could limit the disease-producing steps or reduce symptoms in dengue infected patients. Thus, the development of reagents specific to dengue virus without generation of undesirable adverse effects to intervene the pathogenesis in dengue infected patient will lead to a novel therapeutic approach. In this project, we will apply molecular and synthetic biology to produce human single chain antibody variable fragments (HuScFv) specific to dengue virus proteins and test their binding and inhibiting activities to the corresponding antigens with an ultimate objective to produce therapeutic molecules for treatment of dengue infection.

Research Activities and Results in the Past Three Years

We generated HuScFv with inhibitory effect to dengue virus (DENV) infection. HuScFv molecules were screened and selected from the human antibody phage display library by using purified recombinant DENV NS1 (rNS1), full-length envelope (FL-E) and its domain III (EDIII) proteins as target antigens for bio-panning. HuScFv from two phagemid transformed *E. coli* clones, i.e., clones 11 and 13, bound to the rNS1 as well as native NS1 in both secreted and intracellular forms. Culture fluids of the HuScFv11/HuScFv13 exposed DENV2 infected cells had significant reduction of the infectious viral particles, implying that the antibody fragments affected the virus morphogenesis or release. HuScFv epitope mapping by phage mimotope searching revealed that HuScFv11 bound to amino acids 1-14 of NS1, while the HuScFv13 bound to conformational epitope at the C-terminal portion of the NS1 (66). EDIII-specific HuScFv15A exhibited neutralizing effect to DENV infection in Vero cells in a dose-dependent manner as determined by plaque formation and cell ELISA. Epitope mapping and molecular docking results concordantly revealed interaction of HuScFv to functional loop structure in EDIII of the DENV E protein (67). Although the functions of the epitopes and the molecular mechanism of the HuScFvs further investigations, these small antibodies have high potential for development as anti-DENV biomolecules.

In addition, HuScFv antibodies that bound specifically to macrophage migration inhibitory factor (MIF) were produced and tested activities. MIF is a pro-inflammatory cytokine, secreted from a variety of immune cells that regulates innate and adaptive immune responses. Elevated levels of MIF have been detected in sepsis as well as numerous infectious diseases with exaggerated immune response such as dengue hemorrhagic fever. The rMIF-specific HuScFv with highest binding signal to rMIF also inhibited the tautomerase activities of both rMIF and native MIF in human monoblastic leukemia (U937) cells in a dose-dependent manner. Mimotope searching and molecular docking concordantly demonstrated that the HuScFv interacted with Lys32 and Ile64 in the MIF tautomerase active site. To the best of our knowledge, this is the first study to focus on MIF-specific fully-human antibody fragment with a tautomerase-inhibitory effect that has potential to be developed as anti-inflammatory biomolecules for human use (68).

Furthermore, we used molecular docking to search for a safe anti-DENV drug. The short peptides targeting to the hydrophobic pocket on DENV E protein; a structural transition in the membrane fusion in DENV infection process, were identified. The information of predicted ligand-binding site of reported active compounds to DENV2 hydrophobic pocket was also used for peptide inhibitors selection. The di-peptide, EF, was the most effective on DENV2 infection inhibition in vitro with a half maximal inhibition concentration (IC50) of 96 μM. Treatment of DENV2 with EF at the concentration of 200 μM resulted in 83.47% and 84.15% reduction of viral genome and intracellular E protein, respectively. Among four DENV serotypes, DENV2 was the most effective for the inhibition. Our results provide the proof-of-concept for development of therapeutic peptide inhibitors against DENV infection by the computer-aided molecular design (69).

Other Molecular Biology Research Works

In collaboration with other research groups, we were also involved in the studies of other genetic diseases, such as Wilson disease (WD) and thalassaemia, and other organisms. WD is an autosomal recessive disorder of copper transport characterized by excessive copper deposition in the liver and brain. It is caused by defects of *ATP7B* encoding a copper transporting P-type ATPase. Mutations of *ATP7B* result in lacking of transportation of ceruloplasmin thereby accumulating copper in liver and other tissues such as brain and cornea. Most patients are compound heterozygotes, possessing two alleles with different mutations. To identify the mutations in *ATP7B* in Thai patients with WD, DHPLC analysis was applied to detect mutations and polymorphisms of the entire *ATP7B* gene in 19 Thai patients with WD. Mutations in *ATP7B* were identified in 14 of 19 patients: 2 homozygotes, 8 compound heterozygotes and 4 heterozygotes. Eighteen mutations distributed throughout the entire coding region of *ATP7B* gene including 11 missense, 3 nonsense, 1 splice-site, 1 deletion and 2 insertions. Of 18 different mutations identified, 6 were found to be novel. Twelve single nucleotide polymorphisms (SNPs) were also identified and two SNPs have not yet previously been reported. Segregation analysis using DHPLC analysis showed mutation transmission patterns within each family of Thai patients with WD. Mutations in *ATP7B* in Thai patients with WD are worth adding into the public database for genetic epidemiology and population genetics (70).

Thalassaemia is a genetic disorder affecting the amount of haemoglobin chain production. Thalassaemia syndromes can be classified as being either alpha- and beta-thalassaemia according to the affected globin chains. In beta-thalassaemia, the absence of beta-haemoglobin results in an excess of complementary alpha-haemoglobin whose precipitation leads to oxidative stress, red cell membrane damage, short red cell life span, and ineffective erythropoiesis. An increase in foetal haemoglobin (Hb F) levels in beta-thalassaemia has been shown to ameliorate the disease severity. During the last few decades, foetal haemoglobin reactivation has been considered as a promising intervention to treat β-thalassaemia and sickle-cell anaemia. Variable foetal haemoglobin (HbF, $\alpha 2\gamma 2$) production among individuals is under control of distinct chromosome regions, namely, the quantitative trait loci (QTL). One of the QTL affecting Hb F levels on chromosome 8q has never been explored. The *zinc fingers* and *homeodomains 2* (*ZHX2*) transcription factor located at 8q position is remarkably down-regulated in hereditary persistence of foetal haemoglobin, indicating an inverse correlation between the γ-globin and ZHX2 expression. Here, we studied the effect of ZHX2 over the γ-globin expression in K562 erythroleukaemic cell line by transient transfection with a *ZHX2* expression plasmid. At 24 h after transfection, the relative expression of endogenous γ-*globin* mRNA had been reduced to 0.401 ± 0.08 as assessed by real-time PCR. Our finding suggests that the repressive effect on γ-globin probably results from the presence of ZHX2 and supports its possible involvement in the regulation of γ-globin expression (71).

Our group also studied in helminths and helminth-derived products for their potential to prevent colitis complications in experimental animal and human trials. Helminths use various mechanisms to avoid host immunity and protect themselves from being eliminated. Despite evading host immune responses, immunosuppression and regulation mechanisms elicit functions that diminish the adverse effects of unrelated inflammatory diseases. We investigated whether helminthic infections can ameliorate inflammatory diseases. Mice were infected with *Trichinella papuae* and then subjected to induced colitis through the oral administration of dextran sulfate sodium (DSS). Macroscopic and microscopic examinations measured weight loss, stool consistency, gross bleeding, colon length, and tissue inflammation. In addition, cytokine expression was observed in colon tissue by SYBR real-time RT-PCR to investigate the Th1, Th2, and regulatory cytokines. The results showed that *T. papuaeinfection* decreased the severity of DSS-induced colitis, including weight loss, bloody diarrhea, shortening of colon, and colon tissue damage in mice ($p < 0.05$). The expression level of *IL-4* was high in the colons of

DSS-treated mice without helminthic infection, while infected mice with DSS treatment had lower *IL-4* levels ($p < 0.05$). Uninfected DSS-treated mice failed to produce *IL-10* mRNA in colon tissue, which may cause more severe colitis. In contrast, prior *T. papuae* infection DSS-treated mice had *IL-10* levels in the colon significant lower than the normal and infected control groups. Our data provide the evidence that prior *T. papuae* infection can ameliorate DSS-induced colitis in mice and may be considered for a novel therapeutic strategy against immunological diseases in the future (72).

Angiostrongylus cantonensis infection is the major cause of eosinophilic meningitis. Successful migration and evasion of the immune system by infective-stage larvae (L3) rely heavily on secreted proteases which activate human pro-matrix metalloprotease (MMP-9) into active MMP-9. This study showed that the proteases in excretory-secretory (ES) products of *A. cantonensis* third stage larvae degraded recombinant and native human proMMP-9 in a dose- and time- dependent manner. Protease inhibitory assays showed that metalloproteases were the key enzymes involved in the degradation of human proMMP-9. To assess the effects of ES products on inflammation, ES products were incubated with THP-1 human monocytic cells, which showed induction of MMP-2 and not MMP-9 production. These results indicated that degradation of MMP-9 was due to metalloproteases present in ES of *A. cantonensis* L3, which may be involved in suppressing the host's immune response to allow parasite migration to the host central nervous system (73).

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Outputs:

1. Publications

1.1. Publications in peer-reviewed international journals

*1.1.1. Mutita Junking, Nunghathai Sawasdee, Natapol Duangtum, Boonyarit Cheunsuchon, Thawornchai Limjindaporn, **Pa-thai Yenchitsomanus**. Role of adaptor proteins and clathrin in the trafficking of human kidney anion exchanger 1 (kAE1) to the cell surface. Traffic. March 2014 (In press).*

*1.1.2. Panya A, Bangphoomi K, Choowongkomon K, **Yenchitsomanus P**. Peptide Inhibitors against Dengue Virus Infection. Chem Biol Drug Des. February 2014 (In press).*

*1.1.3. Sreekanth G, Chuncharunee A, Sirimontaporn S, Panaampon J,Srisawat C, Morchang A, Malakar S, Thuwajit P, Kooptiwut S, Suttitheptumrong A, Songprakhon P, Noisakran S, **Yenchitsomanus P**, Limjindaporn T. Role of ERK1/2 Signaling in Dengue Virus-Induced Liver Injury. Virus Res. March 2014 (In press).*

*1.1.4. Saokaew N, Poungpair O, Panya A, Tarasuk M, Sawasdee N, Limjindaporn T, Chaicumpa W, **Yenchitsomanus P**. Human monoclonal single-chain*

antibodies specific to dengue virus envelope protein. Lett Appl Microbiol. 2014;58(3):270-7.

1.1.5. *Poungpair O, Bangphoomi K,Chaowalit P, Sawasdee N, Saokaew N, Choowongkomon K, Chaicumpa W,* ***Yenchitsomanus P****. Generation of human single-chain variable fragment antibodies specific to dengue virus non-structural protein 1 that interfere with the virus infectious cycle. mAbs. 2014;6(2):1-9.*

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1.1.10. *Suttitheptumrong A, Khunchai S, Panaampon J, Yasamut U, Morchang A, Puttikhunt C, Noisakran S, Haegeman G,* ***Yenchitsomanus P****, Limjindaporn T. Compound A, a dissociated glucocorticoid receptor modulator, reduces dengue virus-induced cytokine secretion and dengue virus production. Biochem Biophys Res Commun. 2013 Jun 28;436(2):283-8.*

1.1.11. *Nagila A, Netsawang J, Suttitheptumrong A, Morchang A, Khunchai S, Srisawat C, Puttikhunt C, Noisakran S,* ***Yenchitsomanus P****, Limjindaporn T. Inhibition of p38MAPK and CD137 signaling reduce dengue virus-induced TNF-α secretion and apoptosis. Virol J. 2013 Apr 4;10:105.*

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1.1.16. *Khositseth S, Bruce LJ, Walsh SB, Bawazir WM, Ogle GD, Unwin RJ, Thong MK, Sinha R, Choo KE, Chartapisak W, Kingwatanakul P, Sumboonnanonda A, Vasuvattakul S, **Yenchitsomanus P**, Wrong O. Tropical distal renal tubular acidosis: clinical and epidemiological studies in 78 patients. QJM. 2012 Sep;105(9):861-77.*

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1.1.21. *Jungtrakoon P, Plengvidhya N, Tangjittipokin W, Chimnaronk S, Salaemae W, Chongjaroen N, Chanprasert K, Sujjitjoon J, Srisawat C, **Yenchitsomanus P**. Novel adiponectin variants identified in type 2 diabetic patients reveal multimerization and secretion defects. PLoS One. 2011;6(10):e26792.*

1.1.22. *Duangtum N, Junking M, Sawasdee N, Cheunsuchon B, Limjindaporn T, **Yenchitsomanus P**. Human kidney anion exchanger 1 interacts with kinesin family member 3B (KIF3B). Biochem Biophys Res Commun. 2011 Sep 16;413(1):69-74.*

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1.1.27. *Sawasdee N, Junking M, Ngaojanlar P, Sukomon N, Ungsupravate D, Limjindaporn T, Akkarapatumwong V, Noisakran S,* ***Yenchitsomanus P****. Human kidney anion exchanger 1 interacts with adaptor-related protein complex 1 μ1A (AP-1 mu1A). Biochem Biophys Res Commun. 2010;401(1):85-91.*

1.1.28. *Panya A., Sawasdee N., Srisawat C.,* ***Yenchitsomanus P****, Peerapittayamongkol C. Expression of zinc finger and homeobox 2 in erythroleukaemic cells and gamma-globin expression. ScienceAsia. 2010;36 (4), pp. 342-345.*

1.2. Publications in peer-reviewed national journals

1.2.1. *Choochai Nettuwakul, Nanyawan Rungroj, Nunghathai Sawasdee, Nirinya Sudtachat and* ***Pa-thai Yenchitsomanus****. Detection of SNP rs5896 in prothrombin gene by polymerase chain reaction and high-resolution melting analysis. Thai Journal of Genetics. 2013;6(1):54-59.*

1.2.2. *Wiyada Wongwiwat, Apichaya Niyomchan,* ***Pa-thai Yenchitsomanus****, Sirinush Sricharoenvej and Thawornchai Limjindaporn. Depleted immunoglobulin heavy chain binding protein (BiP) expands the endoplasmic reticulum and the Golgi apparatus in dengue virus-infected cells. Siriraj Med J. 2012;64(S1):S91-92.*

2. Proceedings

2.1. Proceedings in peer-review journals

2.1.1. *Sittideth Sangnual, Nanyawan Rungroj, Choochai Nettuwakul, Nunghathai Sawasdee, Suchai Sritippayawan, Duangporn Chuawattana and* ***Pa-thai Yenchitsomanus****. Analysis of SCN10A Gene Mutation in Northeastern Thai Patients with Nephrolithiasis by High Resolution Melting Method. Thai Journal of Genetics. 2013;S1:87-90.*

2.1.2. *Supanun Butkaew, Watip Tangjittipokin, Nalinee Chongjaroen, Kanjana Chanprasert,* ***Pa-thai Yenchitsomanus*** *and Nattachet Plengvidhya. Study of Common Variations of CDKN2A/2B, CDKAL1, HHEX, KCNQ1, MTNR1B, SLC30A8, TCF7L2 and UBE2E2 and Type 2 Diabetes (T2D) in Thais. Thai Journal of Genetics. 2013;S1:167-170.*

2.2. Proceedings in conference book

2.2.1. *Thanyaporn Dechtawewat, Suchada Sengsai, Pucharee Songprakhon, Thawornchai Limjindaporn, Chunya Puttikhunt, Watchara Kasinrerk,* ***Pa-thai Yenchitsomanus****, and Sansanee Noisakran. Association of dengue virus NS1 and hnRNP C1/C2 proteins in different human target cells for dengue virus infection. The 6th International Symposium of The Protein Society of Thailand. Chulabhorn Research Institute Convention Center, Bankok, Thailand. 30 August – 2 September 2011.*

3. Abstracts

3.1. **Abstracts in peer-review international journals**

3.1.1. *Nattachet Plengvidhya, Jatuporn Sujjitjoon, Suwattanee Kooptiwut, Nalinee Chongjarean, Watip Tangjittipokin, and* ***Pa-thai Yenchitsomanus****. A Type 2 Diabetes Associated PAX4 R192H has a Defect in Transcriptional Regulation. Journal of Diabetes Investigation. 2012;3(S1):88*

3.1.2. *Nattachet Plengvidhya, Watip Tangjittipokin, Ninareeman Binnima, Nalinee Chongjarean, Kanjana Chanprasert, Jatuporn Sujjitjoon, Prapaporn Jungtrakoon,* ***Pa-thai Yenchitsomanus****. Genetic study of Maturity Onset*

Diabetes of the Young (MODY) by Linkage Analysis and Exome Sequencing. Journal of Diabetes Investigation. 2012;3(S1):88.

3.1.3. *Nattachet Plengvidhya, Watip Tangjittipokin, Kanjana Chanprasert, Nalinee Chongjaroen, and **Pa-thai Yenchitsomanus**. Replication of Genome-Wide Association Signals of Type 2 Diabetes in Thai Population. A Journal of The Diabetes Association. 2012;61(S1):2687-PO.*

3.1.4. *Almomani EY, Netsawang J,Ngaojanlar P, Sawasdee N, Limjindaporn T, **Yenchitsomanus P**, Malasit P, and Cordat E. Characterizing the role of adaptor protein 1A in the basolateral targeting of kAE1. Biochemistry and Cell Biology. 2011;89(2):275-275.*

3.2. Abstracts in peer-review national journals

3.2.1. *Sreekanth Gopinathan Pillai, Aporn Chuncharunee, Aunchalee Sirimtaporn, Chatchawan Srisawat, **Pa-thai Yenchitsomanus**, Thawornchai Limjindaporn. Role of MAPK's Inhibitors in DENV Mediated Organ Injuries in a Mouse Model. Siriraj Medical Journal. 2013;65(3):A3.*

3.2.2. *Ninareeman Binnima, Nattachet Plengvidhya, Watip Tangjittipokin, Nalinee Chongjarean, Kanjana Chanprasert, Jatuporn Sujjitjoon, Prapaporn Jungtrakoon, **Pa-thai Yenchitsomanus**. Whole-Exome Sequencing (WES) Identified PTCH1 as a Candidate Gene of Maturity-Onset Diabetes of the Young (MODY) in Thais. Siriraj Medical Journal. 2013;65(3):A4.*

3.2.3. *Aussara Panya, Kunan Bangphoomi, Kiattawee Choowongkomon, and **Pa-thai Yenchitsomanus**. Computer-aided Design of Peptide Inhibitors Targeting to Dengue Envelope Protein. Siriraj Medical Journal. 2013;65(3):A5.*

3.2.4. *Sittideth Sangnual, Nanyawan Rungroj, Choochai Nettuwakul, Nunghathai Sawasdee, Suchai Sritippayawan, Duangporn Chuawattana, and **Pa-thai Yenchitsomanus**. High Resolution Melting Analysis for Detection of SCN10A Gene Mutation in Northeastern Thai Patients with Nephrolithiasis. Siriraj Medical Journal. 2013;65(3):A5.*

3.2.5. *Wanthanee Hanchang, Namoiy Semprasert, Thawornchai Limjindaporn, **Pa-thai Yenchitsomanus**, and Suwattanee Kooptiwut. Testosterone Protects Pancreatic β cells AgainstOxidative Stress and Endoplasmic Reticulum (ER) Stress-Induced Apoptosis in Glucotoxicity. Siriraj Medical Journal. 2013;65(3):A15.*

3.2.6. *Suchada Kaewin, Wanthanee Hanchang, Namoiy Semprasert, Thawornchai Limjindaporn, **Pa-thai Yenchitsomanus**, and Suwattanee Kooptiwut. Identification of Genes that Estrogen Prevents Pancreatic β-cell Apoptosis against Glucotoxicity. Siriraj Medical Journal. 2013;65(3):A21.*

3.2.7. *Thanita Thanyaphon, Nattachet Plengvidhya, Watip Tangjittipokin, Nalinee Chongjaroen, Ninareeman Binnima, Kanchana Chanprasert, Jatuporn Sujjitjoon, and **Pa-thai Yenchitsomanus**. Construction and Expression of Possible Pathogenic Hepatocyte Nuclear Factor-1α Mutants Identified in Thai MODY Families. Siriraj Medical Journal. 2013;65(3):A25.*

3.2.8. *Choochai Nettuwakul, Nanyawan Rungroj, Nunghathai Sawasdee, Nirinya Sudtachat and **Pa-thai Yenchitsomanus**. Detection of SNP rs5896 in prothrombin gene by polymerase chain reaction and high-resolution melting analysis. Thai Journal of Genetics. 2013;S(1):405*

3.2.9. *Thanakorn Pungsrinont, Choochai Nettuwakul, Nunghathai Sawasdee, Nanyawan Rungroj, and **Pa-thai Yenchitsomanus**. Genome-Wide Association Study of Kidney Stone Disease in Northeastern Thai Population. Thai Journal of Genetics. 2013;S(1):404.*

3.2.10. *Suwattanee Kooptiwut, Wantanee Hanchang, Jatuporn Sujjitjoon, Namoiy Semprasert, **Pa-thai Yenchitsomanus**, and Nattachet Plengvidhya. Pax4 Double Variants, R192H and P321H, Impaired Survival of Pancreatic β-cells Cultured in High Glucose Medium. Siriraj Medical Journal. 2012;64(6):53*

3.2.11. *Rochanawan Sootichote, Peti Thuwajit, **Pa-thai Yenchitsomanus**, and Chanitra Thuwajit. Toll-like Receptor 4-mediated IL-6 Expression in Paclitaxel Resistance of Breast Cancer. Siriraj Medical Journal. 2012;64(6):70*

3.2.12. *Sasiprapa Kunchai, Aroonroong Suttitheptumrong, Umpa Yasamut, Nunghathai Sawasdee, Janjuree Netsawang Atthapan Morchang, Prapaipit Chaowalit, Sansanee Noisakran, **Pa-thai Yenchitsomanus**, and Thawornchai Limjindaporn. A Modulation of RANTES Production by Dengue Virus Nonstructural Protein 5 and Daxx Interaction. Siriraj Medical Journal. 2012;64(6):71.*

3.2.13. *Mutita Junking, Sopit Wongkham, Banchob Sripa, and **Pa-thai Yenchitsomanus**. Genetic Variation of LGALS3 Encoding Galectin-3 Associated with Chemosensitive Cholangiocarcinoma. Siriraj Medical Journal. 2012;64(6):85.*

3.2.14. *Mayuri Tarasuk, Ornnuthchar Poungpair, Duangporn Ungsupravate, Kunan Bangphoomi, Wanpen Chaicimpa, and **Pa-thai Yenchitsomanus**. Human Single-chain Variable Antibody Fragment (HuScFv) Neutralizing Tautomerase Activity of Macrophage Migration Inhibitory Factor (MIF). Siriraj Medical Journal. 2012;64(6):86.*

3.2.15. *Aroonroong Suttitheptumrong, Jutatip Panaampon, Sasiprapa Kunchai, Umpa Yasamut, Mutita Junking, Sansanee Noisakran, Guy Haegeman, **Pa-thai Yenchitsomanus**, and Thawornchai Limjindaporn. Compound A Inhibits Cytokine and Chemokine Secretion of DENVinfected HepG2 Cells. Siriraj Medical Journal. 2012;64(6):105.*

3.2.16. *Amar Nagila, Janjuree Netsawang, Atthapan Morchang,Sansanee Noisakran, Chatchawan Srisawat, **Pa-thai Yenchitsomanus**, and Thawornchai Limjindaporn. Role of p38MAPK Signaling in Dengue Virus-Mediated Apoptosis. Siriraj Med J. 2012;64:A3.*

3.2.17. *Jatuporn Sujjitjoon, Nattachet Plengvidhya, Suwattanee Kooptiwut, Wanthanee Hanchang, Titikan Chukitrungroat, Watip Tangjitipokin, and **Pa-thai Yenchitsomanus**. Paired box 4 Mutants under a High-Glucose Condition. Siriraj Med J. 2012;64:A4.*

3.2.18. *Thanyaporn Dechtawewat, Pucharee Songprakhon, Thawornchai Limjindaporn, Chunya Puttikhunt, Watchara Kasinrerk, **Pa-thai Yenchitsomanus**, and Sansanee Noisakran. Role of hnRNP C1/C2 and Dengue VirusNS1Association in Dengue Virus Production. Siriraj Med J. 2012;64:A4.*

3.2.19. *Wanthanee Hanchang, Namoiy Semprasert, Thawomchai Limchindaporn, **Pa-thai Yenchitsomanus**, and Suwattanee Kooptiwut.Testosterone Protects High Glucose-InducedPancreatic β Cell Apoptosis via Suppression of OxidativeStress and Endoplasmic Stress (ER) Stress. Siriraj Med J. 2012;64:A5.*

3.2.20. *Nanyawan Rungroj, Nirinya Sudtachat, Choochai Nettuwakul,Nunghathai Sawasdee, Oranud Praditsap, PrapapornJungtrakoon, Suchai Sritippayawan, Duangporn Chuawattana, Sombat Borvornpadungkitti, Chagkrapan Predanon, Wattanachai Susaengrat, and **Pa-thai***

Yenchitsomanus. *A Prothrombin Variant (T165M) Associateswith Kidney Stone Disease in Thai Patients. Siriraj Med J. 2012;64:A12.*

3.2.21. *Nattachet Plengvidhya, Watip Tangjittipokin, NinareemanBinnima, Nalinee Chongjarean, Kanjana Chanprasert, JatupornSujjitjoon, Prapaporn Jungtrakoon, and* ***Pa-thai Yenchitsomanus***. *Mutation Analysis of Genes Causing Maturity-Onset Diabetes of the Young (MODY) in Thaisby Next-Generation Sequencing. Siriraj Med J. 2012;64:A17.*

3.2.22. *Aroonroong Suttitheptumrong, Sasiprapa Kunchai, UmpaYasamut, Jutatip Panaampon, Mutita Junking, Sansanee Noisakran,* ***Pa-thai Yenchitsomanus***, *Thawornchai Limjindaporn. Inflammatory Cytokine Genes Expression inDengue Virus-Infected Hepatic Cell Line. Siriraj Med J. 2012;64:A25.*

3.2.23. *Nichapatr Saokaew, Ornnuthchar Poungpair, Nunghathai Sawasdee, Wanpen Chaicumpa, and* ***Pa-thai Yenchitsomanus***. *Production of Human Single Chain Variable Fragments Specific to Dengue Virus EnvelopeProtein. Siriraj Med J. 2012;64:A25.*

3.2.24. *Nanyawan Rungroj, Choochai Nettuwakul, Nirinya Sudtachat, Oranud Praditsap, Thidarat Wongrat, Nunghathai Sawasdee, Suchai Sritippayawan, Duangporn Chuawattana, Sombat Borvornpadungkitti, Chagkrapan Predanon, Wattanachai Susaengrat, and* ***Pa-thai Yenchitsomanus***. *A Whole Genome SNP Genotyping by DNA Microarray and Candidate Gene Association Studyfor Kidney Stone Disease. Siriraj Med J. 2012;64:A26.*

3.2.25. *Thanakorn Pungsrinont, Choochai Nettuwakul, Nunghathai Sawasdee, Nanyawan Rungroj, Suchai Sitippayawan, and* ***Pa-thaiYenchitsomanus***. *Stone Disease in Northeastern Thai Population. Siriraj Med J. 2012;64:A28.*

3.2.26. *Wanthanee Hanchang, Namoiy Semprasert, Thawomchai Limchindapom,* ***Pa-thai Yenchitsomanus****, and Suwattanee Kooptiwutl. Protective effect of testosterone on death of pancreatic beta-cells cultured in high-glucose. Proceedings of the 40th Annual Meeting. Annual Scientific Meeting, The Physiological Society of Thailand, International Conference, Khon Kaen, Thailand, May 2-4, 2011. JPBS Vol. 24, (S1), 2011, ISSN 0857-5754.*

3.2.27. *Pitchnischa Mahawoog, Keerati Waochai, Namoiy Sernprasert, Malika Churiotaraphan, Srnarn Onreabro, Supornpirn Chearsku, Thawornchai Lirnjiodaporn,* ***Pa-thai Yeochisornanus****, and Suwattanee Kooptiwut. Effect of estrogen on INS-1 pancreatic beta-cell apoptosis cultured in high glucose. Proceedings of the 40th Annual Meeting. Annual Scientific Meeting, The Physiological Society of Thailand, International Conference, Khon Kaen, Thailand, May 2-4, 2011. JPBS Vol. 24, (S1), 2011, ISSN 0857-5754.*

3.2.28. *Keerati Wanchai, Pornnipa Mahawong, Namoiy Semprasert, Malika Churintaraphan, Smam Onreabroi, Supornpim Chearslrul, Chatchawan Srisawat,* ***Pa-thai Yenchisomanus****, and Suwattanee Kooptiwut. Effect of estrogen on angiotensin II type 1 receptor expression in pancreatic beta-cell prolonged culture in high glucose. Proceedings of the 40th Annual Meeting. Annual Scientific Meeting, The Physiological Society of Thailand, International Conference, Khon Kaen, Thailand, May 2-4, 2011. JPBS Vol. 24, (S1), 2011, ISSN 0857-5754.*

4. Presentations

4.1. Presentations in international conferences

4.1.1. Sasiprapa Khunchai, Mutita Junking, Aroonroong Suttitheptumrong, Suwattanee Kooptiwut, Guy Haegeman, Thawornchai Limjindaporn, and ***Pa-thai Yenchitsomanus****. Dengue virus nonstructural protein 5 enters the nucleus to activate RANTES production via NF-κB. Keystone Symposia on Molecular and Cellular Biology, Emerging Cytokine Networks and the joint meeting on Inflammatory Diseases: Recent Advances in Basic and Translational Research and Therapeutic Treatments, Fairmont Hotel Vancouver, Vancouver, British Columbia, Canada, 17-22 January 2014. (Poster)*

4.1.2. Thanyaporn Dechtawewat, Pucharee Songprakhon, Thawornchai Limjindaporn, Chunya Puttikhunt, Watchara Kasinrerk, ***Pa-thai Yenchitsomanus*** *and Sansanee Noisakran. A potential contribution of hnRNP C1/C2 and dengue virus NS1 association to dengue virus infection. The Third International conference on Dengue and Dengue Haemorrahagic fever "Global Dengue: Challenges and Pomises", The Imperial Queen's Park Hotel, Bangkok, Thailand. 21-23 October 2013. (Poster)*

4.1.3. Kenneth Hodge, Chairat Tunghirun, Pipatpong Temyarasilp, Thawornchai Limjindaporn, ***Pa-thai Yenchitsomanus*** *and Sarin Chimnaronk. Exploration of the dengue RdRp-RNA interface essential for viral replication. The Third International conference on Dengue and Dengue Haemorrahagic fever "Global Dengue: Challenges and Pomises", The Imperial Queen's Park Hotel, Bangkok, Thailand. 21-23 October 2013. (Oral)*

4.1.4. Umpa Yasamut, Mutita Junking, ***Pa-thai Yenchitsomanus*** *and Thawornchai Limjindaporn. Adaptor Protein Complex-1 Involves in Post-Entry Steps of Dengue Virus Life Cycle. CEID 10th Annual Scientific Symposium, Stanley Ho Centre for Emerging Infectious Diseases Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, 21-22 October 2013. (Oral)*

4.1.5. Sreekanth Gopinathan Pillai, Aporn Chuncharunee, Aunchalee Sirimtaporn, Chatchawan Srisawat, ***Pa-thai Yenchitsomanus****, and Thawornchai Limjindaporn. Role of MAPK's Inhibitors in DENV Mediated Organ Injuries in a Mouse Model. Academic Research Presentaion & Graduate Research Forum 2013 - International Conference in Medicine and Publish Health (ICMPH) 2013 "Healthy Society Beyond Frontiers". Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand. 24-28 June 2013. (Oral)*

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4.1.31. *Santi Maneewatchararangsri, Poom Adisakwattana, Urai Chaisri, Potjanee Srimanote and Pongrama Ramasoota. Construction of IL-17 receptor-green fluorescent fusion proteins (IL-17R-GFP) for in vitro ligand-receptor binding fluorescent assay. Joint International Tropical Medicine Meeting 2010 (JITMM2010) "Tropical Diseases: future threats and new paradigms" and International Malaria Colloquium 2010 (IMC2010) "Malaria: new hopes, new challenges". Centara Grand & Bangkok Convention Centre at CentralWorld, Bangkok, Thailand. 1-3 December 2010. (Poster)*

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4.2. Presentations in national conferences

4.2.1. Shilu Malakar, Jutatip Panaampon, ***Pa-thai Yenchitsomanus****, Thawornchai Limjindaporn. Extracellular Signal-Regulated Kinase Inhibitor Inhibits Dengue Virus Production in HepG2 Cells. การประชุมวิชาการเสนอผลงานวิจัยระดับบัณฑิตศึกษา ครั้งที่ 15: 50 ปี แห่งการอุทิศเพื่อสังคม บัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น. วันที่ 28 มีนาคม 2557. (Poster)*

4.2.2. Thawornchai Limjindaporn, Sasiprapa Khunchai, Aroonroong Suttitheptumrong, Mutita Junking, Jutatip Panaampon, Sansanee Noisakran, ***Pa-thai Yenchitsomanus****. The Role of Dengue Virus Nonstructural Protein 5 in Cytokine Production. การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 13. โรงแรมเดอะรีเจนท์ ชะอำบีช รีสอร์ท หัวหิน ชะอำ จ.เพชรบุรี. วันที่ 16-18 ตุลาคม 2556. (Oral)*

4.2.3. Sansanee Noisakran, Thanyaporn Dechtawewat, Pucharee Songprakhon, Thawornchai Limjindaporn, Sittiruk Roytrakul, Atchara Paemanee, Watchara Kasinrerk, Prida Malasit, and ***Pa-thai Yenchitsomanus****. Interactions of Human Host Cellular Proteins with the Dengue Virus NS1 Protein. การประชุมนักวิจัยรุ่นใหม่ พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 13. โรงแรมเดอะรีเจนท์ ชะอำบีช รีสอร์ท หัวหิน ชะอำ จ.เพชรบุรี. วันที่ 16-18 ตุลาคม 2556. (Oral)*

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4.2.9. *Atthapan Morchang, Jutatip Panaampon,* ***Pa-thai Yenchitsomanus*** *and Thawornchai Limjindaporn. The Role of Cathepsin in Dengue Virus-induced Apoptosis. RGJ – Ph.D. Congress XIV. Jomtien Palm Beach Resort, Pattaya, Chonburi, Thailand. 5-7 April 2013. (Poster)*

4.2.10. *Umpa Yasamut, Mutita Junking,* ***Pa-thai Yenchitsomanus*** *and Thawornchai Limjindaporn. Depletion of Adaptor Protein Complex-1μ Subunit Alters Localization of Envelope Protein and Reduces Dengue Virus Production. RGJ – Ph.D. Congress XIV. Jomtien Palm Beach Resort, Pattaya, Chonburi, Thailand. 5-7 April 2013. (Poster)*

4.2.11. *Nattachet Plengvidhya. Novel Adiponectin Variants Identified in Type 2 Diabetic Patients Reveal Multimerization and Secretion Defects. PLoS One. 2011;6(10):e26792.งานประชุมวิชาการประจำปี ครั้งที่ 28 ราชวิทยาลัยอุรแพทย์แห่งประเทศไทย อาคารเฉลิมพระบารมี 50 ปี. 28 เมษายน 2555. (Oral)*

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4.2.16. *Chaiyadol Tantasith, Sansanee Noisakran, Suwatanee Kuptiwut,* ***Pa-thai Yenchitsomanus****, and Thawornchai Limjindaporn. The Role of Dengue Virus Envelope Proteins in Cellular Apoptosis. การประชุมวิชาการกายวิภาคศาสตร์*

แห่งประเทศไทย ครั้งที่ 34. ณ ภาควิชากายวิภาคศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลา-นครินทร์. จังหวัดสงขลา. วันที่ 27-29 เมษายน 2554. (Oral)

4.2.17. ***Pathai Yenchisomanus***. *การวิจัยและประยุกต์จีโนมิกส์ทางการแพทย์และเภสัชศาสตร์. การประชุมวิชาการพันธุศาสตร์ ครั้งที่ 17 เรื่อง การวิจัยพันธุศาสตร์เพื่อแปรผลสู่การประยุกต์. ณ โรงแรมอิมพีเรียลแม่ปิง จังหวัดเชียงใหม่. 7 - 9 เมษายน 2554. (Oral)*

4.2.18. ***Pa-thai Yenchitsomanus***. *Distal Renal Tubular Acidosis: From Genetic to Functional Studies. The 50th Anniversary of Siriraj Nephology Academic Meeting "Highlights in Nephrology 2010". จังหวัดชลบุรี. วันที่ 22-25 ตุลาคม 2553. (Oral)*

4.2.19. ***Pa-thai Yenchitsomanus***. *พันธุศาสตร์และอณูชีววิทยาของโรคนิ่วในไต. การประชุมหารือทิศทางงานวิจัยโรคไต. ณ อาคารสวทช. (โยธี) กระทรวงวิทยาศาสตร์และเทคโนโลยี กรุงเทพ. วันที่ 9 ธันวาคม 2553. (Oral)*

4.2.20. ***Pa-thai Yenchitsomanus***. *Chairperson. การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. ครั้งที่ 10. โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ จังหวัดเพชรบุรี. วันที่ 14-16 ตุลาคม 2553. (Oral)*

4.2.21. *Wanna Thongnoppakhun, Duangkamon Bunditworapoom, Manop Pithukpakorn, Kriengsak Vareesangthip,* ***Pa-thai Yenchitsomanus****, and Chanin Limwongse. First Report of PKD2 Mutations in Thai ADPKD Patients Using Denaturing High Performance Liquid Chromatography (DHPLC) Analysis. การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. ครั้งที่ 10. โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ จังหวัดเพชรบุรี. วันที่ 14-16 ตุลาคม 2553. กันยายน2553. (Poster)*

4.2.22. *Watip Tangjittipokin, Nalinee Chongjarean, Nattachet Plengvidhya, Napatawn Banchuin, and* ***Pa-thai Yenchitsomanus***. *Genetic Polymorphisms of Transcription Factor 7-Like 2 (TCF7L2) are Associated with Type 2 Diabetes in Thai Population. การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. ครั้งที่ 10. โรงแรมฮอลิเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ จังหวัดเพชรบุรี. วันที่ 14-16 ตุลาคม 2553. (Poster)*

4.2.23. *Natapol Duangtum, Mutita Junking, Nunghathai Sawasdee, Thawornchai Limjindaporn, and* ***Pa-thai Yenchitsomanus***. *The Role of Kinesin Family Member 3B (KIF3B) in Trafficking of Human Kidney Anion Exchanger 1 (kAE1). Commission on Higher Education Congress III University Staff Development Consortium (CHE-USDC Congress III). Royal Cliff Grand Hotel and Spa. จังหวัดชลบุรี. วันที่ 9-11 กันยายน 2553. (Poster)*

4.2.24. *Sasiprapa Khunchai, Thawornchai Limjindaporn, Wiyada Wongwiwat, Nunghathai Sawasdee, Prapaipit Chaowalit, Pucharee Songprakhon, and* ***Pa-thai Yenchitsomanus***. *Cytokine production and Inflammatory Responses Induced by Dengue Virus Nonstructural Protein 5. Commission on Higher Education Congress III University Staff Development Consortium (CHE-USDC Congress III). Royal Cliff Grand Hotel and Spa. จังหวัดพัทยา. วันที่ 9-11 กันยายน 2553. (Poster)*

6. Awards

6.1. *Miss Shilu Malakar นักศึกษาปริญญาโท ของ รศ.ดร.นพ. ถาวรชัย ลิ้มจินดาพร ได้รับรางวัลการนำเสนอผลงานแบบโปสเตอร์ ระดับดีเด่น ในการประชุมวิชาการเสนอผลงานวิจัย ระดับบัณฑิตศึกษา ครั้งที่ 15 ณ มหาวิทยาลัยขอนแก่น วันที่ 28 มีนาคม 2557.*

6.2. *Mr. Amar Nagila นักศึกษาปริญญาเอก ของ รศ.ดร.นพ. ถาวรชัย ลิ้มจินดาพร ได้รับรางวัลวิทยานิพนธ์ดีเด่น เรื่อง "The Apoptotic Role of CD137 Signaling in Dengue Virus-infected HepG2 Cells" ใน โครงการรางวัลวิทยานิพนธ์ดีเด่น ประจำปี 2556, บัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล, 2556.*

6.3. *Miss Umpa Yasamut ได้รับรางวัล The winner ของการนำเสนอผลงานวิจัยแบบปากเปล่า เรื่อง Adaptor Protein Complex-1 Involves in Post-Entry Steps of Dengue Virus Life Cycle. ในงานประชุม CEID 10th Annual Scientific Symposium, Stanley Ho Centre for Emerging Infectious Diseases Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, 21-22 October 2013.*

6.4. *Ms. Ninareeman Binnima ได้รับรางวัลที่ 1 ของการนำเสนอผลงานวิจัยแบบปากเปล่า เรื่อง "Whole-Exome Sequencing (WES) Identified PTCH1 as a Candidate Gene of Maturity-Onset Diabetes of the Young (MODY) in Thais" ในงานประชุม International Conference in Medicine and Publish Health (ICMPH) 2013 "Healthy Society Beyond Frontiers". Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand. 24-28 June 2013.*

6.5. *Ms. Aussara Panya ได้รับรางวัลที่ 3ของการนำเสนอผลงานวิจัยแบบปากเปล่า เรื่อง "Computer-aided Design of Peptide Inhibitors Targeting to Dengue Envelope Protein" ในงานประชุม International Conference in Medicine and Publish Health (ICMPH) 2013 "Healthy Society Beyond Frontiers". Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand. 24-28 June 2013.*

6.6. *Mr. Sittideth Sangnua ได้รับรางวัลที่ 3ของการนำเสนอผลงานวิจัยแบบปากเปล่า เรื่อง "High Resolution Melting Analysis for Detection of SCN10A Gene Mutation in Northeastern Thai Patients with Nephrolithiasis. ในงานประชุม International Conference in Medicine and Publish Health (ICMPH) 2013 "Healthy Society Beyond Frontiers". Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand. 24-28 June 2013.*

6.7. *Ms. Thanita Thanyaphon ได้รับรางวัลที่ 2 ของการนำเสนอผลงานวิจัยแบบโปสเตอร์ เรื่อง "Construction and Expression of Possible Pathogenic Hepatocyte Nuclear Factor-1α Mutants Identified in Thai MODY Families" ในงานประชุม International Conference in Medicine and Publish Health (ICMPH) 2013 "Healthy Society Beyond Frontiers". Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand. 24-28 June 2013.*

6.8. *Ms. Wanthanee Hanchang ได้รับรางวัลที่ 3 ของการนำเสนอผลงานวิจัยแบบโปสเตอร์ เรื่อง "Testosterone Protects Pancreatic β cells AgainstOxidative Stress and Endoplasmic Reticulum (ER) Stress-Induced Apoptosis in Glucotoxicity" ในงานประชุมInternational Conference in Medicine and Publish Health (ICMPH) 2013 "Healthy Society Beyond Frontiers". Faculty of Medicine, Mahidol University, Bangkok 10700, Thailand. 24-28 June 2013.*

6.9. *นายสิทธิเดช แสงนวล ได้รับรางวัลผลงานทางวิชาการดีเด่น เพื่อนำเสนอในแบบบรรยาย เรื่อง "การวิเคราะห์การกลายพันธุ์ของยีน SCN10A ในผู้ป่วยโรคนิ่วไตในภาคตะวันออกเฉียงเหนือของไทยด้วยวิธี high resolution melting" ใน การประชุมวิชาการพันธุศาสตร์แห่งชาติ ครั้งที่ 18 (National Genetics Conference 2013)"พันธุศาสตร์ก้าวหน้าสู่อาเซียน" (Genetics toward ASEAN) ณ โรงแรมแอมบาสเดอร์ สุขุมวิท กรุงเทพมหานคร วันที่ 17 – 19 กรกฎาคม 2556.*

6.10. *นางสาวศุภนันท์ บุตรแก้ว ได้รับรางวัลผลงานทางวิชาการ แบบโปสเตอร์ เรื่อง "การศึกษาความแปรผันของยีน CDKN2A/2B, CDKAL1, HHEX, KCNQ1,MTNR1B, SLC30A8, TCF7L2 และUBE2E2 กับการเกิดโรคเบาหวานชนิดที่สองในประชากรไทย" ใน การประชุมวิชาการพันธุศาสตร์แห่งชาติ ครั้งที่ 18 (National Genetics Conference 2013)"พันธุศาสตร์ก้าวหน้าสู่อาเซียน" (Genetics toward ASEAN) ณ โรงแรมแอมบาสเดอร์ สุขุมวิท กรุงเทพมหานคร วันที่ 17 – 19 กรกฎาคม 2556.*

6.11. *นางสาวธัญญาพร เดชทวีวัฒน์ ได้รับรางวัลที่ 2 ของการนำเสนอผลงานวิจัยแบบปากเปล่า เรื่อง "Role of hnRNP C1/C2 and Dengue Virus NS1Association in Dengue Virus Production. ในงานประชุม Graduate Research Forum 2012. International Conference in Medicine and Public Health 2012 (ICMPH2012). From Royal Initiatives towards Healthy Thai Society. 150th Anniversary of the Birth of Queen Sri Savarindira, คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิยาลัยมหิดล วันที่ 18-19 กันยายน 2555.*

6.12. *Mr. Amar Nagila ได้รับรางวัลที่ 2 ของการนำเสนอผลงานวิจัยแบบปากเปล่า เรื่อง "Role of p38MAPK Signaling in Dengue Virus-Mediated Apoptosis". ในงานประชุม Graduate Research Forum 2012. International Conference in Medicine and Public Health 2012 (ICMPH2012). From Royal Initiatives towards Healthy Thai Society. 150th Anniversary of the Birth of Queen Sri Savarindira, คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิยาลัยมหิดล วันที่ 18-19 กันยายน 2555.*

6.13. *นายอัฐพันธ์ หมอช้าง นักศึกษาปริญญาโท ได้รับรางวัลวิทยานิพนธ์ ระดับดี เรื่อง "Apoptotic Gene Expression Profiling in Dengue Virus-Infected HepG2 Cells" ในโครงการรางวัลวิทยานิพนธ์ดีเด่น ประจำปีการศึกษา 2554 ในวันที่ 19 พฤษภาคม 2555 ณ อาคารเอนกประสงค์ มหาวิทยาลัยมหิดล ศาลายา.*

6.14. *รศ.ดร. นพ. ณัฐเชษฐ์ เปล่งวิทยา ได้รับรางวัล Research Hilights ในการประชุมวิชาการประจำปี ครั้งที่ 28 ราชวิทยาลัยอายุรแพทย์แห่งประเทศไทย ณ โรงแรมแอมบาสเดอร์ซิตี้ จอมเทียน จังหวัดชลบุรี ในวันที่ 28 เมษายน 2555.*

6.15. *ศ.ดร. เพทาย เย็นจิตโสมนัส ได้รับรางวัลบุคลากรดีเด่น คณะแพทยศาสตร์ศิริราชพยาบาล ประจำปี 2554 ในวันศุกร์ที่ 24 กุมภาพันธ์ 2555 ณ หอประชุมราชแพทยาลัย คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล*

7. Student Graduation

7.1. Doctoral degree

7.1.1.	*Student name*	*Miss Wanthanee Hanchang*
	Thesis title	*Identification of testosterone effects in pancreatic beta cell prolonged cultured in high glucose condition*
	Date of graduation	*February 27, 2014*
	Advisory committee	*Assoc. Prof. Supatra Lohsiriwat, M.D.* *Assoc. Prof. Suwattanee Kooptiwut, M.D., Ph.D.*

		Prof. Pa-thai Yenchitsomanus, Ph.D. *Assoc. Prof. Supornpim Chearskul, M.D.* *Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.* *Assoc. Prof. Nattachet Plengvidhya, M.D.* *Assoc. Prof. Thiti Snabboon, M.D.*
7.1.2.	*Student name* *Thesis title* *Date of graduation* *Advisory committee*	*Miss Sasiprapa Khunchai* *Nuclear localization of dengue virus nonstructural protein 5: The induction of RANTES production by activation of NF- κB* *October 1, 2013* *Assoc. Prof. Nattiya Hirankarn, M.D., Ph.D.* *Prof. Pa-thai Yenchitsomanus, Ph.D.* *Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.* *Asst. Prof. Potjanee Srimanote, Ph.D.* *Lect. Sansanee Noisakran, Ph.D.*
7.1.3.	*Student name* *Thesis title* *Date of graduation* *Advisory committee*	*Mr. Sreekanth Gopinathan Pilai* *Role of extracellular signal-regulated kinase (ERK) in a mouse model of dengue virus-mediated liver injury* *August 13, 2013* *Asst. Prof. Kanitha Patarakul, M.D., Ph.D.* *Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.* *Prof. Aporn Chuncharunee, D.V.M., MS.* *Asst. Prof. Chatchawan Srisawat, M.D., Ph.D.* *Asst. Prof. Peti Thuwajit, M.D., Ph.D.*
7.1.4.	*Student name:* *Thesis title* *Date of graduation* *Advisory committee*	*Mr. Amar Nagila* *The apoptotic role of CD137 signaling in dengue virus-infected HepG2 cells* *October 26, 2012* *Asst. Prof. Kanitha Patarakul, M.D., Ph.D.* *Assoc. Prof Thawornchai Limjindaporn, M.D., Ph.D.* *Prof. Pa-thai Yenchitsomanus, Ph.D.* *Asst. Prof. Chatchawan Srisawat, M.D., Ph.D.* *Assoc. Prof. Wannee Kantakamalakul, Ph.D.*
7.1.5.	*Student name:* *Thesis title:* *Date of graduation:* *Advisory committee:*	*Miss Prapaporn Jungtrakoon* *Adipocentin (ADIPOQ) variants identified in Thai patients with type 2 diabetes* *June 17, 2011* *Prof. Pa-thai Yenchitsomanus, Ph.D.* *Assoc. Prof. Nattachet Plengvidhya, M.D.* *Asst. Prof. Chatchawan Srisawat, M.D.,Ph.D.*

7.2. Master degree

7.2.1.	*Student name:* *Thesis title:* *Date of graduation:* *Advisory committee:*	*Mr. Sittideth Sangnual* *Analysis of voltage-gated sodium channel type 10 gene mutation in Northeastern Thai patients with nephrolithiasis* *November 26, 2013* *Assoc Prof. Sookkasem Khositseth, M.D., Ph.D.*

Prof. Pa-thai Yenchitsomanus, Ph.D.
Lect. Wanna Thongnoppakhun, Ph.D.
Lect. Nanyawan Rungroj, Ph.D.
Assoc. Prof. Varaporn Akkarapatumwong, Ph.D.

7.2.2. Student name: *Mrs. Aroonroong Suttitheptumrong*
Thesis title: *Inhibition of inflammatory cytokine production in dengue virus-infected HepG2 cells by compound A*
Date of graduation: *August 5, 2013*
Advisory committee: *Asst. Prof. Kanitha Patarakul, M.D., Ph.D.*
Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.
Prof. Pa-thai Yenchitsomanus, Ph.D.
Lect. Sansanee Noisakran, Ph.D.

7.2.3. Student name: *Miss Thanyaporn Dechtawewat*
Thesis title: *Role of hnRNP C1/C2 and dengue virus NS1 association in dengue virus infection*
Date of graduation: *July 31, 2013*
Advisory committee: *Asst. Prof. Kanitha Patarakul, M.D., Ph.D.*
Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.
Prof. Pa-thai Yenchitsomanus, Ph.D.
Lect. Sansanee Noisakran, Ph.D.

7.2.4. Student name: *Miss Nichapatr Saokaew*
Thesis title: *Human single chain variable antibody fragments specific to envelope protein domain III inhibit dengue virus serotype 2 infection*
Date of graduation: *May 17, 2013*
Advisory committee: *Asst. Prof. Potjanee Srimanote, Ph.D.*
Prof. Pa-thai Yenchitsomanus, Ph.D.
Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.
Prof. Wanpen Chaicumpa, Ph.D.

7.2.5. Student name: *Mr. Chaiyadol Tuntasit*
Thesis title: *The role of dengue virus envelop protein in cellular apoptosis*
Date of graduation: *May 10, 2012*
Advisory committee: *Assoc. Prof. Thawornchai Limjindaporn, M.D., Ph.D.*
Prof. Pa-thai Yenchitsomanus, Ph.D.
Assist. Prof. ChatchawanSrisawat, M.D., Ph.D.

7.2.6. Student name: *Miss Pitchnischa (Pornnipa) Mahawong*
Thesis title: *The effect of estrogen on oxidative stress and endoplasmic reticulum stress (ER stress) in impaired pancreatic beta-cell function with high glucose*
Date of graduation: *October 18, 2011*
Advisory committee: *Assoc. Prof. Suwattanee Kooptiwut, M.D., Ph.D.*
Assoc. Prof. Supornpim Chearskul, M.D.
Prof. Pa-thai Yenchitsomanus, Ph.D.
Assist. Prof. Panapat Uawithya, M.D., Ph.D.

7.2.7. Student name: *Miss Keerati Wanchai*
Thesis title: *Effect of estrogen on angitensin II receptor type 1 expression in impaired pancreatic beta-cell function with high glucose*

Date of graduation: *October 18, 2011*
Advisory committee: *Assoc. Prof. Suwattanee Kooptiwut, M.D., Ph.D.*
Assoc. Prof. Supornpim Chearskul, M.D., Ph.D.
Prof. Pa-thai Yenchitsomanus, Ph.D.
Assist. Prof. Chatchawan Srisawat, M.D., Ph.D.

7.2.8. Student name: *Mr. Pongpet Benjaponwattana*
Thesis title: *The association of MGP gene and renal stone formation.*
Date of graduation: *June 2, 2011*
Advisory committee: *Apinunt Udomkit, Ph.D.*
Varaporn Akkarapatumwong, Ph.D.
Pa-thai Yenchitsomanus, Ph.D.
Surapon Piboonpocanun, Ph.D.
Nattiya Hirankarn, M.D., Ph.D.
Wanna Thongnoppakhun, Ph.D.
Nanyawan Rungroj, Ph.D.

8. Current students

8.1. Doctoral degree

8.1.1. Miss Oranud Praditsap
8.1.2. Miss Nalin-on Nuiplot
8.1.3. Miss Jatuporn Sujjitjoon
8.1.4. Mr. Natapol Duangtum
8.1.5. Miss Aussara Panya
8.1.6. Miss Patta Phumesin
8.1.7. Mr. Atthapan Morchang
8.1.8. Miss Umpa Yasamut
8.1.9. Miss Nopparat Thongmueng
8.1.10. Miss Shilpa Lekshmi Leela
8.1.11. Miss Chutamas Thepmalee
8.1.12. Miss Liji Sreelatha

8.2. Master degree

8.2.1. Miss Rochanawan Sootichote
8.2.2. Miss Thidarat Rattanaburee
8.2.3. Miss Thanita Thanyaphon
8.2.4. Miss Kamonpat Supimon
8.2.5. Miss Shilu Malakar
8.2.6. Mr. Arnat Pasena

Conference arrangements

1. *Human Genomics and Molecular Biology 2013 on July 17-19, 2013, at Ambassador Hotel, Bangkok, Thailand.*

   The topics of lectures included:
   1. *Overview on Research Activities of TRF Senior Research Scholar Group by Professor Pa-thai Yenchitsomanus, Division of Molecular Medicine, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.*
   2. *Next Generation Sequencing Technology in Genomic Research by Dr. Natini Jinawath, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand.*

3. *Next Generation Sequencing Data Analysis and Interpretation by Dr. Bhoom Suktitipat, Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol Unviserty, Thailand.*
4. *Genetic Association Study of Kidney Stone Disease in Northeastern Thai Population by Dr. Nanyawan Rungroj, Division of Molecular Genetics, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol Unviserty,Thailand.*
5. *Identification of Gene Causing Kidney Stone Disease in Northeastern Thai Families by Linkage Analysis and Exome Sequencing by Miss Oranud Praditsap, Division of Molecular Genetics, Faculty of Medicine Siriraj Hospital, Mahidol Unviserty, Thailand*
6. *Genetics and Molecular Biology of Distal Renal Tubular Acidosis (dRTA) by Dr. Mutita Junking, Division of Molecular Medicine, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.*
7. *Genetics and Clinical Intervention of Diabetes by Assoc. Prof. Nattachet Plengvidhya, Division of Endocrinology and Metabolism, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.*
8. *Molecular Genetic Study of Diabetes by Dr. Watip Tangjittipokin, Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.*
9. *Role of PAX4 in the Regulation of Pancreatic Beta cell Apoptosis by Jatuporn Sujjitjoon, Department of Immunology and Immunology Graduate Program, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand*

2. *Molecular Medicine Conference (MMC2012) "Alternative Strategies againist Cancer and Inflammation" on December 19-22, 2012, at Srisavarindira Building, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

The topics of lectures included:

1. *Virchow Explained: The Origin of Tumor Elicited Inflammation and ItsSignificance by Professor Michael Karin, University of California, San Diego, USA.*
2. *$P28^{GANK}$, A Novel Target for Hepatocellular Carcinomas by Professor Hongyang Wang, Eastern Hepatobiliary Surgery Institute/Hospital, Shanghai, P.R. China.*
3. *Inflammatory Control of Colorectal Cancer Progression by Professor Yinon Ben-Neriah, Hebrew University, Jerusalem, Israel.*
4. *Mitochondria in Innate Immunity by Professor Sankar Ghosh, Columbia University, New York, USA.*
5. *Dual Regulatory Roles of Syk in TLR4 Signaling and Inflammatory Response by Professor Wan-Wan Lin, National Taiwan University, Taipei, Taiwan.*
6. *New Functions of Non-canonical IKKs by Professor M. Lienhard Schmitz, University of Giessen, Giessen, Germany.*
7. *Oncoproteomic Analysis Reveals Co-upregulation of RELA and STAT5 in Carboplatin Resistant Ovarian Carcinoma by Dr. Natini Jinawath, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand.*

8. *The Roles of the MAPK-activated Protein Kinases (MKs) in Inflammation, Cancer and Beyond by Professor Matthias Gaestel, Hannover Medical University, Hannover, Germany.*
9. *Roles of Lipid Oxidation in Liver Fluke-induced Cholangiocarcinoma by Assoc. Prof. Puangrat Yongvanit, Faculty of Medicine, KhonKaen University, Thailand.*
10. *T Cell Development and Immunotherapy by Professor Lung-Ji Chang, University of Florida, Florida, USA.*
11. *Applied Systems Biology for the Control of Metastatic Cancer by Professor Albrecht Reichle, University Hospital Regensburg, Regensburg, Germany.*
12. *Targeting Cancer-Stromal Fibroblast Interaction as a Potential Therapeutic Strategy by Asst. Prof. Chanitra Thuwajit, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.*
13. *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring by Professor Anton Amann, Breath Research Institute, Austrian Academy of Sciences, Austria.*
14. *Inflammation-associated Carcinogenesis and Its Chemoprevention with Bioactive Natural Substances by Professor Young-Joon Surh, Seoul National University, Seoul, South Korea.*
15. *Natural Compounds as Inhibitors of the 10 Hallmarks of Cancer by Assoc. Prof. Marc Diederich, LBMCC, Kirchberg Hospital, Luxemburg, Germany.*
16. *Mangifera indica Stem Bark Extract (Vimang) and Its Main Polyphenol Mangiferin: From the Ethnomedicine as Natural Supplements to the Preclinical and Clinical Investigations for New Phytomedicines by Professor Rene Delgado, Drug Research and Development Centre, Havana, Cuba.*
17. *Essential Role for Cardiomyocyte Glucocorticoid Receptors in the Prevention of Heart Disease by Professor John Cidlowski, NIEHS/NIH, North Carolina, USA.*
18. *New Molecular Mechanisms Involved in the Immune Actions of Glucocorticoids by Professor Eduardo Arzt, Institute of the Max Planck Society, Buenos Aires, Argentina.*
19. *Tissue-specific Action of Glucocorticoids by Assoc. Prof. Hirotoshi Tanaka, Institute of Medical Science, University of Tokyo, Japan.*
20. *The Origin and History of Compound A by Professor Pieter Swart, University of Stellenbosh, Stellenbosh, South Africa.*
21. *A Desert Plant-derived Compound against Inflammation by Professor Guy Haegeman, LEGEST, University of Gent, Belgium.*
22. *Anti-cancer Potential of Selective Glucocorticoid Receptor Activators: A Novel Approach to GR-targeted Chemotherapy by Assoc. Prof. Irina Budunova, Northwestern University, Chicago, USA.*
23. *Molecular Mechanisms for Corticosteroids Resistance and Its Reversal by Professor Peter Barnes: Imperial College London, UK.*
24. *Epigenetic Alterations and Cancer Chemoprevention by Dietary Polyphenols by Professor Ajay Goel, Baylor University Medical Center, Dallas, USA.*
25. *The Epigenetic Regulation of Autophagy in Cancer Cells and Its Impact on Chemotherapy: Role of Stroma and Inflammation by Professor Ciro Isidoro, A. Avogadro University, Novara, Italy.*
26. *The Consequences of Genome Wide Hypomethylation in cis by Professor Apiwat Mutirangura, Faculty of Medicine, Chulalongkorn University, Thailand.*

27. *Inflammation Signaling in Skin Carcinogenesis by Professor Zigang Dong, The Hormel Institute, University of Minnesota, USA.*
28. *Glucocorticoid Stress Hormones and Resilience to Brain Disease by Professor Ron de Kloet, LACDR/LUMC, Leiden University, The Netherlands.*
29. *Functional Foods in Diet-induced Metabolic Syndrome by Professor Lindsay Brown, University of Southern Queensland, Toowoomba, Australia.*
30. *The Protective Ability of Anatolian Plant Extracts against Glycoxidation and Oxidative Protein Damage by Professor Cimen Karasu, Faculty of Medicine, Gazi University, Ankara, Turkey.*
31. *15-Hydroxyprostaglandin Dehydrogenase as a Novel Molecular Target for Chemoprevention of Inflammation-associated Carcinogenesis by Asst. Prof. Hye-Kyung Na, Sungshin Women's University, Seoul, South Korea.*
32. *Genome-wide Survey of Recurrent HBV Integration in Hepatocellular Carcinoma to Survey Hepatitis B Virus (HBV) Integration in Liver Cancer Genomes by Assoc. Prof. Sung Wing Kin, Illumina, Inc.*

3. *การประชุมวิชาการโครงการเมธีวิจัยอาวุโส ประจำปี 2554 ในวันที่ 30 พฤษภาคม 2554 ณ ห้องบงกชรัตน์ เอ โรงแรมรอยัลริเวอร์ กรุงเทพฯ*

หัวข้อการบรรยายประกอบด้วย:

1. *Biomolecular crosstalk in breast cancer โดย รศ. ดร. พิมพิชฌา ปัทมสิริวัฒน์ ภาควิชาจุลทรรศนศาสตร์คลินิก คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล*
2. *Genetic study of renal stone disease โดย นางสาวอรนุช ประดิษฐ์ทรัพย์ หลักสูตรวิทยาภูมิคุ้มกัน ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
3. *Genome-wide association study of renal calculi by DNA microarray โดย นายนายชูชัย เนตรธุวกุล หน่วยอณูเวชศาสตร์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
4. *Helminth therapy: new prospect against inflammatory diseases? โดย อ. ดร. ภูมิ อดิศักดิ์วัฒนา ภาควิชาปรสิตหนอนพยาธิ คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล*
5. *Computer-aided drug design: A case study of virtual screening of Thai medicinal plants against the activity of tyrosine kinase (TK) domain of EGFR for cancer therapy โดย ผศ. ดร. เกียรติทวี ชูวงศ์โกมล ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์*
6. *Genomic studies of Thai type 2 diabetes โดย รศ. นพ. ณัฐเชษฐ์ เปล่งวิทยา ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
7. *Novel adiponectin variants identified in type 2 diabetic patients reveal multimerization and secretion defects โดย นางสาวประภาพร จึงตระกูล หลักสูตรวิทยาภูมิคุ้มกัน ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
8. *Identification of copy number variation (CNV) in CAPN10 gene by denaturing high pressure liquid chromatography (dHPLC) โดย นางกาญจนา ชาญประเสริฐ ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
9. *Role of CD137 signaling in dengue virus-mediated apoptosis โดย รศ. ดร. นพ. ถาวรชัย ลิ้มจินดาพร ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*

10. *Cell death gene expression profile: role of RIPK2 in dengue virus-mediated apoptosis* *โดย นางสาวอำภา ยาสมุทร์ หลักสูตรวิทยาภูมิคุ้มกัน ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
11. *Production of human single chain variable fragments specific to dengue virus envelope protein* *โดย นางสาวฉิชาภัทร เสาแก้ว หลักสูตรวิทยาภูมิคุ้มกัน ภาควิชาวิทยาภูมิคุ้มกัน คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
12. *Generation of dengue capsid protein-specific human transbody* *โดย นางสาวประไพพิศ เชาวลิต หน่วยอณูเวชศาสตร์ สถานส่งเสริมการวิจัย คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
13. *Single variable light chain (VL) antibody to human interleukin 17A (IL-17A) and IL-17F heterodimers: therapeutic strategy for blocking bioactivity* *โดย อ. ดร. สันติ มณีวัชระรังษี ภาควิชาชีวโมเลกุลและพันธุศาสตร์โรคเขตร้อน คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล*
14. *Effect of sex hormones on pancreatic β–cell survival after prolonged culture in high glucose* *โดย รศ. ดร. พญ. สุวัฒณี คุปติวุฒิ ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
15. *Effect of estrogen on ER stress reduction in pancreatic β-cells after prolonged culture in high glucose* *โดย นางสาวพิชญ์ณิชชา มหาวงศ์ หลักสูตรสรีรวิทยา ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*
16. *Effect of estrogen on angiotensin II type 1 receptor expression in pancreatic β-cell prolonged culture in high glucose* *โดย นางสาวกีรติ วันชัย หลักสูตรสรีรวิทยา ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ศิรราชพยาบาล มหาวิทยาลัยมหิดล*

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(Pa-thai Yenchitsomanus)
Principal Investigator