

List of RGJ advisors 2023/2024

(14)

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Keywords:	kidney injury, epigenetics, DNA damage, mgmt, mouse model		
Summary of research:	Anti-inflammatory epigenetic alteration in innate immune cells through blockage of DNA damage repair methyl-guanine methyl transferase (MGMT) will be explored both in vivo (renal ischemia model) and in vitro (macrophages and neutrophils) using LyM-Cre <i>mgmt</i> conditional depletion mice versus wild type mice.		

- การกรอกรายละเอียดในแบบฟอร์มนี้ ต้องดำเนินการให้ครบถ้วนตามความเป็นจริง หากตรวจสอบพบว่ามีกรปิดหรือเป็นเท็จ วิชา. ขอสงวนสิทธิ์ที่จะไม่พิจารณาสนับสนุนและจะเป็นผู้ไม่มีสิทธิ์รับทุน วิชา. เป็นเวลา ๓ ปี

แบบเสนอโครงการวิจัย (Research Project)

ประกอบการเสนอขอทุนอุดหนุนการวิจัยของสำนักงานการวิจัยแห่งชาติ (วช.)

โครงการปริญญาเอกกาญจนาภิเษก (คปก.) ภายใต้ความร่วมมือไตรภาคีไทย-สวีเดน

ประจำปีงบประมาณ ๒๕๖๗

๑. ชื่อโครงการวิจัย (ภาษาไทย) การยับยั้งการซ่อมแซมดีเอ็นเอของ myeloid cells เพื่อลดความรุนแรงของภาวะการอักเสบของไตจากการขาดเลือดโดยใช้หนูที่ขาด *mgmt* โดยระบบ LysM-Cre เพื่อค้นหาข้อบ่งชี้ของสารยับยั้ง MGMT
(ภาษาอังกฤษ) Defect on DNA damage repair of myeloid cells in ischemic reperfusion injury using conditional *mgmt*-deleted LysM-Cre mice, the possible impacts of MGMT Inhibitor in kidney disease
๒. ชื่อ-สกุล อาจารย์ที่ปรึกษา อัสภาศ ลีฬหวนิชกุล (Asada Leelahavanichkul)
หน่วยงาน สาขามิคุ้มกันวิทยา ภาควิชา จุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย (Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University)
สถานที่อยู่ที่ติดต่อดีสะดวก ตึก อปร. ชั้น 17 รพ. จุฬาลงกรณ์ สภากาชาดไทย (17th floor Aor Por Ror Building, King Chulalongkorn Memorial Hospital) พร้อมหมายเลขโทรศัพท์ โทรศัพท์มือถือ 081-714-9750 โทรสาร และไปรษณีย์อิเล็กทรอนิกส์ aleelahavanit@gmail.com
๓. กลุ่มสาขาวิทยาศาสตร์พื้นฐานที่สมัคร (เลือกเพียง ๑ กลุ่ม)
 ชีววิทยา (Biology) เคมี (Chemistry)
 ฟิสิกส์ (Physics) คณิตศาสตร์ (Mathematics)
๔. ผู้ใช้ประโยชน์ (Research stakeholders) (กรณีมีความร่วมมือ) เช่น ความร่วมมือของหน่วยงานภาครัฐ (เช่น กระทรวง กรม)/เอกชนที่ร่วมสนับสนุนทุนวิจัย เช่น MOU เป็นต้น
 มี..... ไม่มี
๕. คำสำคัญ (Keyword):

methylguanine methyltransferase, macrophage, neutrophil, LysM-Cre mice

๖. ความสำคัญและที่มาของปัญหาที่ทำการวิจัย (Problem statement and significance of research)

Acute kidney injury (AKI) is one of the important healthcare problems worldwide with a significant economic loss due to the induction of chronic kidney disease and renal replacement therapy (1). The most common cause of AKI is renal ischemia from several causes that induce inflammation against the necrotic cells mostly by innate immune cells, especially macrophages and neutrophils (1). The inflammatory responses from these myeloid innate immune cells against the renal tissue cause renal damage and exacerbate the progression of fibrosis in kidney leading to end stage renal condition and fastening the initiation of renal replacement therapy. Although the best supportive treatment is the only main theme of current AKI management, several novel anti-inflammatory strategies (2, 3, 4) are interesting which, at least some, some of them might be able to attenuate inflammation-induced renal injury. The delay progression of AKI into chronic kidney disease (CKD) and postponing the need for renal replacement therapy will be benefit to the patients and the economic status of the country. The O6-methylguanine-DNA methyltransferase (MGMT) is a DNA suicide repair enzyme and the blockage of MGMT might reduce functions of innate immune cells enough to attenuate renal damage of ischemia-induced kidney injury.

๗. ทฤษฎี/สมมุติฐานของโครงการ (Hypothesis)

Blockage of DNA repair in myeloid cells (macrophages and neutrophils) attenuates damages in kidney during acute kidney injury (AKI).

๘. วัตถุประสงค์ของโครงการ (Objectives)

๘.๑ To compare the severity of acute kidney injury (AKI) in LysM-Cre *mgmt* conditional deletion mice versus wild type mice

๘.๒ To see the possible impact of *mgmt*. inhibitor on AKI attenuation

๙. การทบทวนวรรณกรรม/ผลงานวิจัยที่เกี่ยวข้อง (Literature Review)

Anti-inflammatory epigenetic alteration in innate immune cells through blockage of DNA damage repair methyl-guanine methyl transferase (MGMT)

Several manipulations for controlling functions of innate immune cells (macrophage and neutrophil), have been mentioned (5), including the control through epigenetic change (an alteration in the gene translation processes without an alteration in the genetic code through the modification level on histone, DNA, and RNA (6). DNA damage repair (DDR) pathways maintain cell function after stress-induced DNA methylation that produces N⁷-methylated purines and O⁶-methylguanine (O⁶MeG) (5, 6). The methylated DNA bases are removed by base excision repair initiated by the alkyladenine-DNA glycosylase, the family of AlkB homologs proteins, and the suicidal enzyme O⁶-methylguanine-DNA methyltransferase (MGMT) (5, 6). MGMT blockage is a powerful inducers of cell death via the formation of O⁶MeG in cancer cells and MGMT blockage in macrophages causes anti-inflammation in sepsis mouse models (5, 6). However, the possible use of MGMT blockage which is clinically available for cancer treatment in other pro-inflammatory conditions is also possible.

Inflammation worsens renal damage after ischemic acute kidney injury

Ischemic reperfusion injury is a common cause of AKI through a relatively low blood perfusion to kidney from several causes, including drugs, hypovolemic shock, and distributive shock (sepsis and anaphylaxis). Innate immune cells, especially macrophages and neutrophils, are responsible for the post-ischemic damages through several mechanisms; such as M1-pro-inflammation and neutrophil extracellular traps (NETs) (1, 2, 7). Hence, the blockage of innate immune cells might be possible to attenuate post-ischemic renal damages. Interestingly, LysM-Cre *mgmt* deletion mice allowing the exploration of mice with the *mgmt* depletion only in myeloid cells (macrophages and neutrophils), but not other cell types, leading to a more solid conclusion on impacts of *mgmt* than the use of *mgmt* knock-out mice.

๑๐. ระเบียบวิธีวิจัย (Methodology)

1. Ischemic reperfusion injury model

Male 8-wk-old mice from LysM-Cre *mgmt* deletion mice and wild type group will be operated for ischemia reperfusion injury according to an established protocol (1). At 24 or 48 h later, mice will be sacrifice through cardiac puncture under isoflurane anesthesia with

sample collection (blood and organs). Organs (kidney, liver, and lung) will be explored for histological injury using hematoxylin and eosin color and the fluorescent colors (Ly6G, F4/80, myeloperoxidase, neutrophil elastase) to see the accumulation of macrophages, neutrophils, and neutrophil extra cellular traps (NETs) formation. In parallel, organs will be used to explore gene expression by polymerase chain reaction and Western blot analysis of several inflammatory pathways.

2. Macrophage functions

Bone marrow derive macrophages will be extracted from the long bones according to an established protocol (4). Then, the macrophages from *mgmt*-depleted and WT mice will be activated by a representative uremia toxin (indoxyl sulfate) and/ or lipopolysaccharide with or without MGMT blocker to see the possible different responses, especially M1 and M2 macrophage polarization by PCR (iNOS, IL-1b, arginase, etc.), flow cytometry analysis (CD86 and CD206), and supernatant cytokines.

3. Neutrophil functions

Neutrophils will be extracted from *mgmt*-depleted and WT mice using thioglycolate-based method as previously described (1) or magnetic cell sorting. The cells will be activated as similar to the macrophage experiments to explores NETosis, cytokines, and gene expression by PCR.

๑๑. ขอบเขตของการวิจัย (Scope of the study); in vivo and in vitro experiments using LyM-Cre *mgmt* conditional deletion mice versus wild type group.

๑๒. ผลผลิต (Output) ผลลัพธ์ (Outcome) และ ผลกระทบ (Impact) ที่คาดว่าจะได้จากการวิจัย

Output and outcome: the new knowledge on the extended indication to use MGMT blockage in non-cancer conditions. Also, at least 2 international publications (higher than Q1) will be generated from this proposal.

Impact: the use of MGMT blockage will change the current management of AKI that currently have only a supportive treatment.

References

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CURRICULUM VITAE

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Educations:

1990-1996 M.D. (honors), Chulalongkorn University, Thailand
 2002 Board of Internal Medicine, Thailand
 2003 Board of Family Medicine, Thailand
 2004 Board of Nephrology, Thailand
 Master of Science, Chulalongkorn University, Thailand
 2005-2010 Visiting fellow at National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Bethesda, MD, USA
 2010 PhD (Biomedical Science), Chulalongkorn University, Thailand
 2019 Board of Clinical Pathology, Thailand

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Chulalongkorn University, Bangkok, Thailand	M.D.	1996	General doctor
Chulalongkorn University, Bangkok, Thailand	M.D. (specialty)	2002	Internal Medicine
Chulalongkorn University, Bangkok, Thailand	M.D. (specialty)	2003	Family Medicine
Chulalongkorn University, Bangkok, Thailand	M.S. and M.D. (specialty)	2004	Nephrology
Chulalongkorn University, Bangkok, Thailand	PhD.	2010	Biomedical Science
Chulalongkorn University, Bangkok, Thailand	M.D. (specialty)	2019	Clinical pathology

Brief Chronology of Employment

2011- present Lecturer, Immunology unit, Department of microbiology, Chulalongkorn University, Thailand
 Invited Lecturer, Division of Nephrology, Department of Medicine,

	Chulalongkorn University, Thailand
2005- 2010	Visiting Fellow, Kidney Disease Branch, National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD, USA
2004-2005	Instructor, Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, Chulalongkorn University, Thailand
1998-1999	General practice, Ongkarag Hospital, Nakornnayok, Thailand
1997-1998	General practice, Savee Hospital, Chumpon, Thailand
1996-1997	Internship, Maharajch Hospital, Nakornsridhammaraj, Thailand

Honors:

2022	Most citation awards in pre-clinical level (2019-2022) from Research Affairs, Faculty of Medicine, Chulalongkorn University
2021	Categorized as an expert in sepsis by "expertscape.com"
2017	2 nd place of award from the Department of Medical Science (DMSc award) in Medical Research on <i>Lactobacillus rhemnosus</i> derived from Thai infant for <i>Clostridium difficile</i> infection treatment
2016	Cerebos Award from Cerebos incorporation (BRAND's) with the study of <i>Lactobacillus rhemnosus</i> derived from Thai infant for sepsis prevention
2016	National Outstanding Researcher Award on Using Animals for Scientific Purpose Development (IAD) from National Research Council of Thailand (NRCT) (รางวัลนักวิจัยดีเด่นที่ใช้สัตว์เพื่องานทางวิทยาศาสตร์ รางวัล สททว. (IAD Award) ประจำปี พท. 2559 โดยสถาบันพัฒนาการดำเนินการต่อสัตว์เพื่องานทางวิทยาศาสตร์ (สททว) สำนักงานคณะกรรมการวิจัยแห่งชาติ (วช.) 2016 Rachadapisak Sompotch award from Chulalongkorn University with the study of NGAI in bilaterat nephrectomy versus bilateral ureter obstruction model
2005	Visiting fellow award, Kidney Disease Branch, National Institute of Diabetes and Digestive and Kidney Disease

Professional Membership and Associations:

The Medical Council of Thailand
The Royal College of Physicians of Thailand
The Nephrology Society of Thailand
The Clinical Pathology Society of Thailand

Publications:

1. **Leelahavanichkul A**, Areepium N, Vadcharavivad S, Praditpornsilpa K, Avihingsanon Y, Karnjanabuchmd T, Eiam-Ong S and Tungsanga K. Pharmacokinetics of Sirolimus in Thai Healthy Volunteers. *J Med Assoc Thai* 2005; **88** Suppl 4: S157-62.
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