2024 Biomedical Research Symposium of National Health Research Institutes

113 年度國家衛生研究院生物醫學學術研討會

Program Book

July 31 - August 1, 2024

※ 本摘要集內容請勿擅自引用 ※

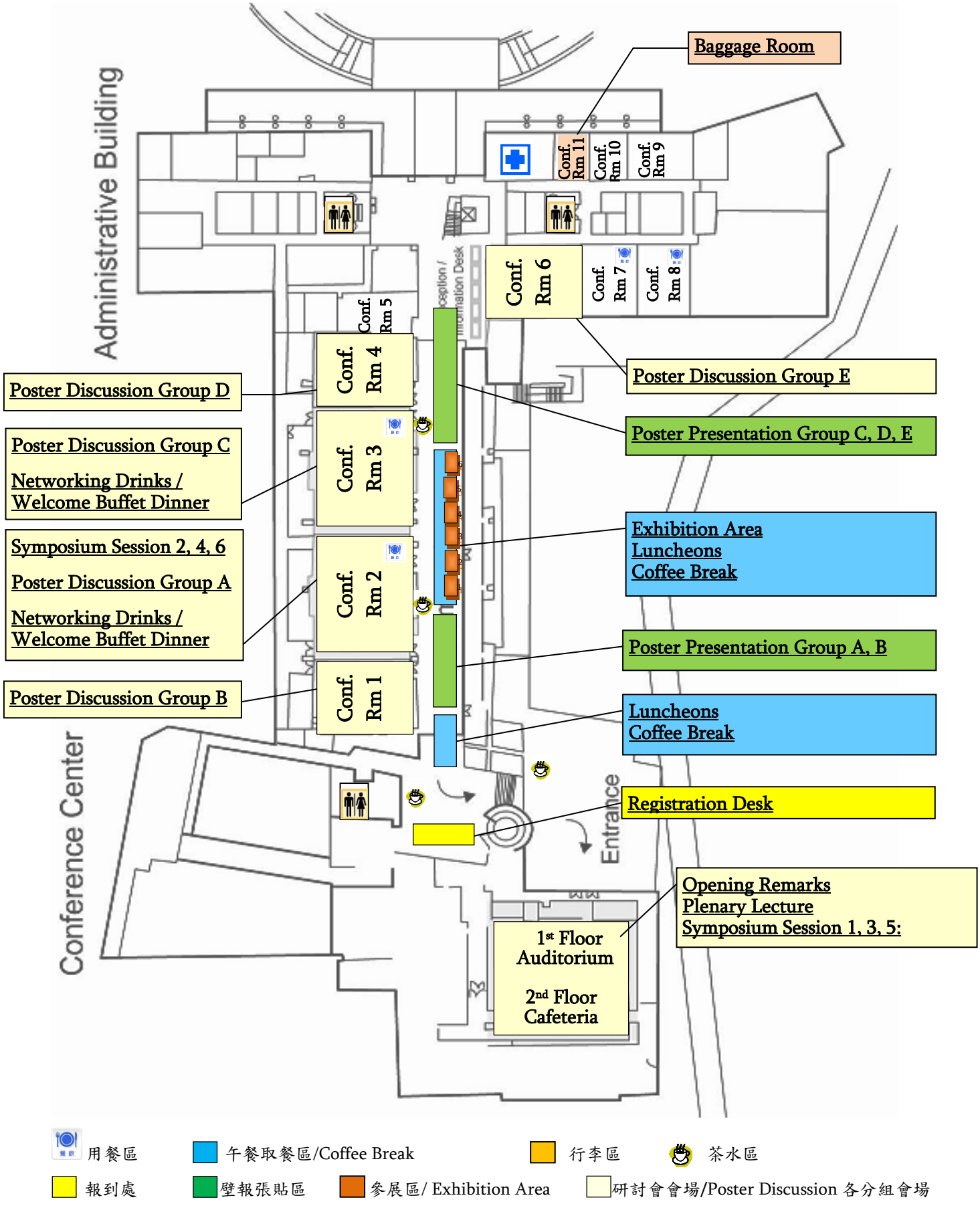
National Health Research Institutes, Taiwan

CONTENTS

PRORGAM AT A GLANCE

	Wednesday, July 31, 2024			Thursday, August 1, 2024	
09:00				09:00-09:50 **Plenary Lecture** (Venue : Auditorium)	09:00
09:30	09:30-10:00 **Registration**				09:30
10:00	10:00-10:10 **Opening Remarks** (Venue : Auditorium)			09:50-10:00 Coffee Break	10:00
10:30	10:10-11:00 **Plenary Lecture** (Venue : Auditorium)			10:00-11:00 **Poster Session 2 even numbers**	10:30
11:00	11:00-11:20 Coffee Break				11:00
11:30	11:20-13:00 **Symposium Session 1** : Cancer Research (Venue : Auditorium)	11:20-13:00 **Symposium Session 2** : Medical Engineering (Venue : Conference Room 2)		11:00-12:40 **Symposium Session 5** Biomedical Science 2 (Venue : Auditorium)	11:30
12:00				11:00-12:40 **Symposium Session 6** Clinical and Public Health (Venue : Conference Room 2)	12:00
12:30					12:30
13:00	13:00-14:00 Lunch			12:40-13:40 Lunch	13:00
13:30					13:30
14:00	14:00-15:40 **Symposium Session 3** : Biomedical Science 1 (Venue : Auditorium)	14:00-15:40 **Symposium Session 4** : Neuroscience (Venue : Auditorium)	14:00-15:40 **Poster Discussion** Group D - Medical Engineering (Venue: Conference Room 4)	13:40-15:40 **Poster Discussion** (Group A-E) (Venue : Conference Room 2, 1, 3, 4, 6)	14:00
14:30					14:30
15:00					15:00
15:30					15:30
	15:40-16:00 Coffee Break				
16:00	16:00-17:00 **Poster Session 1-odd numbers**				16:00
16:30					16:30
17:00	17:00-17:30 Networking Drinks				17:00
17:30	17:30-19:00 Welcome Buffet Dinner				17:30
18:00					18:00
18:30					18:30
19:00					19:00

113 年度國家衛生研究院生物醫學學術研討會
2024 Biomedical Research Symposium of National Health Research Institutes
會場分佈圖 (Floor Plan)



Daily Program

July 31（Wednesday）

09:30-10:00	**Registration**	
10:00-10:10	**Opening Remarks (Venue : Auditorium)** *Dr. Huey-Kang Sytwu* 司徒惠康院長 National Health Research Institutes 國家衛生研究院	
10:10-11:00	**Plenary Lecture (Venue : Auditorium)** Chairperson: *Dr. Kenneth K. Wu* 伍焜玉院士 National Health Research Institutes / Academia Sinica 國家衛生研究院/中央研究院 **Making Universal Vaccines and Antibodies Through Glycoengineering** *Dr. Chi-Huey Wong* 翁啟惠院士 Academia Sinica 中央研究院	
11:00-11:20	**Coffee Break**	
	Symposium Session 1 : Cancer Research (Venue : Auditorium) Chairpersons: *Dr. Tso-Pang Yao* 姚佐邦教授 Duke University *Dr. Wen-Chun Hung* 洪文俊特聘研究員 National Health Research Institutes 國家衛生研究院	**Symposium Session 2 : Medical Engineering (Venue : Conference Room 2)** Chairpersons: *Dr. Zong-Ming Li* 李宗明教授 University of Arizona *Dr. Chia-Wen (Kevin) Wu* 吳嘉文特聘研究員 National Health Research Institutes 國家衛生研究院
11:20-11:45	**Endocrine Organ-Like Tumor Hypothesis: VLDLR Orchestrated Cancer Requiem** *Dr. Wen-Lung Ma* 馬文隆教授 China Medical University 中國醫藥大學	**Elasticity Measurements of 3D Cell Culture Systems: Principles, Devices and Potential Applications to Stiffness-Targeted Treatment Delivery** *Dr. Pai-Chi Li* 李百祺特聘教授 National Taiwan University 國立臺灣大學
1:45-12:10	**From Microtubule Depolymerase to Drug Resistance: The Role of KIF2C** *Dr. Lily Hui-Ching Wang* 王慧菁教授 National Tsing Hua University 國立清華大學	**Ultrafast High Frequency Ultrasound Imaging and Its Biomedical Applications** *Dr. Chih-Chung Huang* 黃執中特聘教授 National Cheng Kung University 國立成功大學

12:10-12:35	**Host-microbiota Interaction in Intestinal Tumorigenesis: Three-way Crosstalks Between Epithelia, Bacteriome, and Virome** *Dr. Linda Chia-Hui Yu* 余佳慧教授 National Taiwan University 國立臺灣大學	**Parathyroid Hormone (PTH) for Osteoarthritis Treatment: from Bench to Pre-clinical** *Dr. Chung-Hwan Chen* 陳崇桓教授 Kaohsiung Medical University 高雄醫學大學
12:35-13:00	**Targeting Extracellular HSP90α as Novel Anti-Desmoplasia and Anti-Cachexia Strategy for Pancreatic Adenocarcinoma Therapy** *Dr. Tze-Sing Huang* 黃智興研究員 National Health Research Institutes 國家衛生研究院	**Deciphering Tissue Microstructures and Neural Connectivity of Postmortem Fetal Brains with High-resolution Connectome MRI** *Dr. Li-Wei Kuo* 郭立威副研究員 National Health Research Institutes 國家衛生研究院
13:00-14:00	**Lunch**	

	Symposium Session 3 : Biomedical Science 1 **(Venue : Auditorium)** Chairpersons: *Dr. Edward T.H. Yeh* 葉篤行院士 University of Arkansas for Medical Sciences *Dr. Cathy (Chiou-Hwa) Yuh* 喻秋華研究員 National Health Research Institutes 國家衛生研究院	**Symposium Session 4 : Neuroscience** **(Venue : Conference Room 2)** Chairpersons: *Dr. Lina Wei* 魏麗娜教授 University of Minnesota *Dr. Wei J. Chen* 陳為堅特聘研究員 National Health Research Institutes 國家衛生研究院	**Poster Discussion (Venue: Conference Room 4)** Chairperson: *Dr. Zong-Ming Li* 李宗明教授 University of Arizona
14:00-14:25	**Precision Health in Childhood Hearing Loss: from Diagnostics, Prognostics to Therapeutics** *Dr. Chen-Chi Wu* 吳振吉教授 National Taiwan University 國立臺灣大學	**Mechanisms of Vascular Dementia--Risk Factors, Glymphatics, Neuro-inflammation, Senescence** *Dr. Chaur-Jong Hu* 胡朝榮教授 Taipei Medical University 臺北醫學大學	**Group D - Medical Engineering**

14:25-14:50	**Distinguishing between Intragenic Trans-spliced and Circular RNAs by Long-read Sequencing** *Dr. Trees-Juen Chuang* 莊樹諄研究員 Academia Sinica 中央研究院	**Unlocking Promise: α6GABA$_A$R Positive Modulators for Neuropsychiatric Disorders** *Dr. Lih-Chu Chiou* 邱麗珠教授 National Taiwan University 國立臺灣大學
14:50-15:15	**Exploring the Metabolic Gene Regulatory Network in Neuroendocrine Prostate Cancer for Potential Immunotherapy Applications** *Dr. Yen-Nien Liu* 劉晏年教授 Taipei Medical University 臺北醫學大學	**Pain Caused by Citric Acid: From Drug Formulations to Animal Venoms and Beyond** *Dr. Ted Weita Lai* 賴威達副教授 China Medical University 中國醫藥大學
15:15-15:40	**The Novel Targets and Combinatorial Therapies for Treatment of the Aggressive Breast Cancer** *Dr. Hsin-Ling Hsu* 徐欣伶研究員 National Health Research Institutes 國家衛生研究院	**Polygenic Prediction of Antidepressant Treatment Response** *Dr. Yen-Feng Lin* 林彥鋒助研究員級主治醫師 National Health Research Institutes 國家衛生研究院
15:40-16:00	**Coffee Break**	
16:00-17:00	**Poster Session 1 - odd numbers**	
17:00-17:30	**Networking Drinks**	
17:30-19:00	**Welcome Buffet Dinner**	

August 1（Thursday）

09:00- 09:50	**Plenary Lecture (Venue : Auditorium)** Chairpersons: *Dr. Ing-Kang Ho* 何英剛院士 Academia Sinica 中央研究院 **Microglial Galectin-3 and Neuroinflammation – with A Specific Focus on Neurodegenerative Diseases** *Dr. Yijuang Chern* 陳儀莊特聘研究員 Academia Sinica 中央研究院	
09:50- 10:00	**Coffee Break**	
10:00- 11:00	**Poster Session 2 - even numbers**	
	Symposium Session 5 : Biomedical Science 2 (Venue : Auditorium) Chairpersons: *Dr. Reen Wu* 吳忍教授 University of California at Davis *Dr. Cheng-Chin Kuo* 郭呈欽研究員 National Health Research Institutes 國家衛生研究院	**Symposium Session 6 : Clinical and Public Health (Venue : Conference Room 2)** Chairpersons: *Dr. C. Kent Kwoh* 郭堅教授 University of Arizona Arthritis Center *Dr. Hung-Yi Chiou* 邱弘毅特聘研究員 National Health Research Institutes 國家衛生研究院
11:00- 11:25	**Microbiota-Derived Metabolites and Cardiac Resilience: Unveiling the Gut-Heart Connection** *Dr. Patrick Ching-Ho Hsieh* 謝清河特聘研究員 Academia Sinica 中央研究院	**Investigation of Care Continuity and Care Coordination for Patients with Multiple Chronic Conditions under the Universal Health Insurance Scheme in Taiwan** *Dr. Shou-Hsia Cheng* 鄭守夏特聘教授 National Taiwan University 國立臺灣大學
11:25- 11:50	**Decoding Precise Immune Regulation and Human Diseases with Multimodal Single-Cell Analysis of Peripheral Immune Perturbations** *Dr. Tai-Ming Ko* 柯泰名副教授 National Yang Ming Chiao Tung University 國立陽明交通大學	**Non-invasive Brain Stimulation in Stroke Rehabilitation and the Application of EEG Markers** *Dr. Ching-Yi Wu* 吳菁宜特聘教授 Chang Gung University 長庚大學

11:50-12:15	**NLRP12, an Innate Immune Checkpoint: Its Regulatory Properties in Pathogen Infections and Lupus Disease** *Dr. Szu-Ting Chen* 陳斯婷副教授 National Yang Ming Chiao Tung University 國立陽明交通大學	**To Elucidate the Interplay of Aldehyde Dehydrogenase 2 and Acrolein in Chronic Kidney Diseases** *Dr. Hsiang-Tsui Wang* 王湘翠教授 National Yang Ming Chiao Tung University 國立陽明交通大學
12:15-12:40	**Exosomes in Stem Cell Homeostasis, Cancer, and Regenerative Medicine** *Dr. Hua-Jung Li* 李華容副研究員 National Health Research Institutes 國家衛生研究院	**Developmental Origin of Atherosclerosis and Neurocognitive Dysfunctions from Environmental Endocrine Disruptors** *Dr. Shu-Li Julie Wang* 王淑麗研究員 National Health Research Institutes 國家衛生研究院
12:40-13:40	**Lunch**	
13:40-15:40	**Poster Discussion (Group A-E)** Chairpersons: **Group A - Biomedical Science (Venue: Conference Room 2)** *Dr. Edward T.H. Yeh* 葉篤行院士 University of Arkansas for Medical Sciences **Group B - Neuroscience (Venue: Conference Room 1)** *Dr. Lina Wei* 魏麗娜教授 University of Minnesota **Group C - Cancer Research (Venue: Conference Room 3)** *Dr. Tso-Pang Yao* 姚佐邦教授 Duke University **Group D - Medical Engineering (Venue: Conference Room 4)** *Dr. Zong-Ming Li* 李宗明教授 University of Arizona **Group E - Clinical and Public Health (Venue: Conference Room 6)** *Dr. Tun-Hou Lee* 李敦厚教授 Harvard TH Chan School of Public Health	

2024 Biomedical Research Symposium of National Health Research Institutes
-Plenary Speakers-

Dr. Chi-Huey Wong, Academia Sinica

Dr. Yijuang Chern, Academia Sinica

Chi-Huey Wong, Ph.D.
Department of Chemistry, The Scripps Research Institute, California
and Genomics Research Center, Academia Sinica
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Phone No.: 02-2789-9929; Fax No.: 02-2789-9927
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Web: https://www.scripps.edu/faculty/wong

Education

1979-1982 Ph.D., Chemistry, MIT

Research and Professional Positions Held in Chronological Sequence

1982-1983	Postdoctoral Fellow, Harvard University
1983-1989	Assistant to Professor of Chemistry (1883-87), Texas A&M University
1989-2006	Ernest W. Hahn Chair Professor of Chemistry, The Scripps Research Institute
1991-1998	Head, Frontier Research Program on Glycotechnology, RIKEN, Japan
2003-2006	Director, Genomics Research Center, Academia Sinica
2006-2019	President, Academia Sinica (2006-16) and Distinguished Research Fellow
2019-present	Scripps Family Chair Professor of Chemistry, The Scripps Research Institute; Distinguished Fellow, Genomics Research Center, Academia Sinica

Major Honors and Awards

1985	Searle Scholar Award in Biomedical Sciences
1986	Presidential Young Investigator in Chemistry
1994	The IUPAC International Carbohydrate Award
1994	Elected Member of Academia Sinica, Taipei
1996	Elected Member of the American Academy of Arts and Sciences
1998	American Chemical Society (ACS) Harrison Howe Award in Chemistry
1999	ACS Claude S. Hudson Award in Carbohydrate Chemistry
1999	The International Enzyme Engineering Award
2000	Presidential Green Chemistry Award, USA
2002	Elected Member of the National Academy of Sciences, USA
2005	ACS Award for Creative Work in Synthetic Organic Chemistry
2008	The F. A. Cotton Medal for Excellence in Chemical Research
2010	Elected Member of the European Molecular Biology Organization (EMBO)
2012	The American Chemical Society Arthur C. Cope Award
2012	Nikkei Asia Prize for Science, Technology and Innovation, Japan
2014	The Wolf Prize in Chemistry
2014	Elected Fellow of the U.S. National Academy of Inventors
2015	The Royal Society of Chemistry Robert Robinson Award, UK
2021	The Welch Award in Chemistry, USA
2022	Chemical Pioneer Award, The American Institute of Chemists
2022	Tetrahedron Prize for Creativity in Organic Synthesis
2023	Barry Cohen International Prize, Israel Chemical Society
2023	Federation of Asian Chemical Societies (FACS) Foundation Lectureship

Making Universal Vaccines and Antibodies Through Glycoengineering

Chi-Huey Wong
翁啟惠

Department of Chemistry, The Scripps Research Institute, USA
Genomics Research Center, Academia Sinica, Taiwan

Biological glycosylation is a process used to modulate the structure and function of biomolecules, particularly those on the surface of cells, and aberrant glycosylation is often found in human pathogens and cancer cells. Most human viruses depend on host glycosylation machinery to create a sugar coat on the viral surface to facilitate infection and escape immune surveillance. The main immunogens of influenza viruses and SARS-CoV-2 are mostly shielded by such host-made sugar coats from immune response, so deletion of the sugar coat would expose the highly conserved epitopes and elicit broadly protective antibody and T cell responses against the virus and different variants. The antibodies induced by such low-sugar vaccines are more diverse with higher titers against the immunogen, especially the highly conserved epitopes, thus broadening the scope of protection. Furthermore, the Fc-glycans on the antibody can be engineered to improve Fc-mediated killing. This strategy has been applied to target cancer-specific glycans for development of broadly protective anti-cancer vaccines and antibodies with improved Fc-mediated killing.

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Education

1980-1984	B.S., Agricultural Chemistry, National Taiwan University, Taiwan
1984-1988	Ph.D., Molecular & Cellular Biology, University of Massachusetts, U.S.A.

Research and Professional Positions Held in Chronological Sequence

1988-1990	Postdoctoral Fellow, Harvard Medical School, U.S.A.
1991-1996	Assistant Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan
1996-2002	Associate Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan
2002-2014	Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan
2014-present	Distinguished Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan
2004-2006	Deputy Director, Institute of Biomedical Sciences, Academia Sinica, Taiwan
2016-2019	Director, Department of International Affairs, Academia Sinica, Taiwan
2022-2024	Deputy Minister, National Science and Technology Council, Taiwan
2024-present	Director, Institute of Biomedical Sciences, Academia Sinica, Taiwan

Research Interests

We focus on two interrelated research projects: (1) functional characterization of the A_{2A} adenosine receptor ($A_{2A}R$) and (2) development of novel therapeutic treatment for degenerative diseases including Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD). $A_{2A}R$ is a major target of caffeine, the most widely used psychoactive substance in the world. My laboratory started from the cloning of this receptor many years ago, and have extend our research interests into the regulation and signal transduction of $A_{2A}R$ under pathophysiological conditions, especially in neurodegenerative diseases. We have also set out to identify and delineate novel pathogeneses (e.g., Galectins-mediated neuroinflammation and abnormal GABAergic signaling) in diseased brains so that better treatments can be designed. In addition, we developed a group of adenosine analogues targeting simultaneously at $A_{2A}R$ and an adenosine transporter (ENT1). These compounds can enter the brain to enhance the adenosine tone and to stimulate adenosine receptors for beneficial signals. We and several other laboratories have demonstrated that these compounds can be used to treat mouse models of at least five protein aggregation diseases (including HD, ALS, SCA3, SCA7, AD, and Niemann-Pick type C disease). These findings have led to the application and approvals of patents on applying these compounds to the development of therapeutic treatments for neurodegenerative diseases.

Major Honors and Awards

1998	Young Investigator Award, Academia Sinica
1998-2000	Member, Scientific Review Committee, Biochemistry/Biology Section, National Science Council, Taiwan
1999-2000	Research Outstanding Award, National Science Council, Taiwan
1999	The eighteenth “Ten Outstanding Young Women”, Taiwan
2001-2002	Research Outstanding Award, National Science Council, Taiwan
2002	The fortieth “Ten Outstanding Young Men”, Taiwan
2002-2004	Research Outstanding Research Award, National Science Council, Taiwan
2014	The Tien Te Lee Biomedical Foundation Outstanding Award, Taiwan
2014-2018	Academia Sinica Investigator Award, Academia Sinica, Taiwan
2019-2023	Academia Sinica Investigator Award, Academia Sinica, Taiwan
2022	侯金堆傑出榮譽獎
2023	第 20 屆國家新創獎

Microglial Galectin-3 and Neuroinflammation –
with A Specific Focus on Neurodegenerative Diseases

Yijuang Chern
陳儀莊

Institute of Biomedical Sciences, Academia Sinica, Taiwan

Microglia are the primary immune cells of the brain and play crucial roles in maintaining brain homeostasis, protecting against infections, and responding to injury. Overactivation of microglia is closely linked to neurodegeneration. Galectin-3 (Gal3), a protein that binds to β-galactoside, has been extensively studied in various peripheral diseases, including lung fibrosis, cancers, and kidney failure. Recently, Gal3 has gained significant attention in neuroscience due to its elevated levels in activated microglia. In this presentation, I will discuss how human iPSC-derived microglia-like cells respond to exposure to pathogenic tau protein—a key factor in neurodegeneration—and their role in propagating this harmful protein in the brain through a Gal3-dependent pathway. Given the importance of microglial activation in many brain disorders, our insights into the pathogenic role of Gal3 could enhance our understanding of microglial responses to misfolded proteins. Additionally, we will discuss the development of Gal3 inhibitors that can enter the brain to treat neurodegenerative diseases involving microglia-driven inflammation.

2024 Biomedical Research Symposium of National Health Research Institutes
-Invited Speakers-

Session 1: Cancer Research

Dr. Wen-Lung Ma, China Medical University
Dr. Lily Hui-Ching Wang, National Tsing Hua University
Dr. Linda Chia-Hui Yu, National Taiwan University
Dr. Tze-Sing Huang, National Health Research Institutes

Session 2: Medical Engineering

Dr. Pai-Chi Li, National Taiwan University
Dr. Chih-Chung Huang, National Cheng Kung University
Dr. Chung-Hwan Chen, Kaohsiung Medical University
Dr. Li-Wei Kuo, National Health Research Institutes

Session 3: Biomedical Science 1

Dr. Chen-Chi Wu, National Taiwan University
Dr. Trees-Juen Chuang, Academia Sinica
Dr. Yen-Nien Liu, Taipei Medical University
Dr. Hsin-Ling Hsu, National Health Research Institutes

Session 4: Neuroscience

Dr. Chaur-Jong Hu, Taipei Medical University
Dr. Lih-Chu Chiou, National Taiwan University
Dr. Ted Weita Lai, China Medical University
Dr. Yen-Feng Lin, National Health Research Institutes

Session 5: Biomedical Science 2

Dr. Patrick Ching-Ho Hsieh, Academia Sinica
Dr. Tai-Ming Ko, National Yang Ming Chiao Tung University
Dr. Szu-Ting Chen, National Yang Ming Chiao Tung University
Dr. Hua-Jung Li, National Health Research Institutes

Session 6: Clinical and Public Health

Dr. Shou-Hsia Cheng, National Taiwan University
Dr. Ching-Yi Wu, Chang Gung University
Dr. Hsiang-Tsui Wang, National Yang Ming Chiao Tung University
Dr. Shu-Li Julie Wang, National Health Research Institutes

Wen-Lung, Ma, PhD

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Education

1991-1997	B.S., Nursing, Chinese Medical College, Taiwan (now China Medical University)
1997-1999	MS., Physiology, National Cheng Kung University, Taiwan
2003-2010	Ph.D., Pathology, University of Rochester, New York, USA

Research and Professional Positions Held in Chronological Sequence

1999-2001	Surgery, Army Medical School (ROTC), Taiwan
2010-2010	Post Doctorate, University of Rochester Memorial Hospital
2011-2017	Assistant Professor, China Medical University
2017-2022	Associate Professor, China Medical University
2022~till now	Professor, China Medical University

Research Interests

My research interests mainly focus on endocrine regulation of tumor, lipid biology, lipidomics, and their roles in disease pathways. We are now working on Liver, Ovary, and Gastric cancers from lipid point of views, conducting translational medical researches. Using transgenic animal, disease animal models, combine modern molecular technology, Omics and bioinformatics, we strived to understand the fundamental of disease, as well as to translate into potential clinical utilizations. The current projects including cancer metabolism, nanomedicine, and substance addiction.

Major Honors and Awards

2007	Student travel award for "The Endocrine Society's 89th Annual Meeting at Toronto", 2007, June 2
2010	Aflac Inc. Young Investigator Award, Travel grant for Scientist-In-Training Award for participating American Association for Cancer Research (AACR) Annual meeting (101st AACR), Washington, D.C., USA
2010	Outstanding Scientific Article Award, Taiwan Liver Diseases Association
2013	The 7th Guangdong and Hong Kong Conference on Liver Disease, Invited Speaker: Title: SEX OR NOT? THAT IS A QUESTION: Translating Androgen Receptor Basic Studies to The Management of Liver Cancer, Guangzhou First Municipal People's Hospital, Guangzhou Medical University, China
2014	指導醫學系學生獲得財團法人臺灣醫學發展基金會論文獎競賽第三名

2015 Distinguished Young Scientist Award grant from Taiwan Ministry Of Sciences and Technology (MOST) for four year continue support. Grant title: Translational Liver Cancer Research: From Androgen Receptor Signal Basic Study to The Evaluation of Therapeutic Potentials

2017 Invited Speaker: 32nd Joint Annual Conference of BioMedical Sciences, Taipei, 25th~26th /Mar, 2017. Title: Pathophysiology of Cancer: The Tumor Macroenvironmental Regulation. Host by the "The Chinese Physiological Society"

2019 Outstanding Professor (Associate Professor), China Medical University

2019 *Ad Hoc grant reviewer: Agence National de la Recherche of France; AAPG2019, Generic Call for Proposal*

2019 Co-funded MOST Shackleton program grant to Academician Luhai Wang of CMU.

2020 Funded by MOST program grant: Innovative Translational Research; Novel Targets in Human Health and Diseases.

2021 Distinguished Research Paper Award, Society of Endocrinology, Edinburg, Scotland, UK, for the paper published in Endocrine-Related Cancer 2020 with title "Low Density Lipoprotein Receptor-Mediated Lipidome-Transcriptome Reprogramming Impulses to Cisplatin Insensitivity"

2022 Three times of NHRI funding award Medal.

2023 2nd TLRS (Taiwan Lipidomics Research Symposium),
Keynote Lecturer: Paradigm Shift of Lipid in BioMedical Sciences

2023 ISREC-SCCL Symposium 2023: Precision Oncology
Selected Abstract: Lipid Scavenging Deficit and Overindulgence Promote Hepatocellular Carcinoma Progression

Title: Endocrine Organ-Like Tumor Hypothesis: VLDLR Orchestrated Cancer Requiem

Wen-Lung Ma, Pei-Yin Liao, Wen-Jen Lin,
馬文隆，廖珮吟，林文仁

Graduate institute of Biomedical Sciences, China Medical University, Taiwan

Since our team proposed EOLT (Endocrine Organ-Like Tumor) hypothesis since 2016, we've strived to prove it. We define EOLT that Tumors Dictate Homeostatic by overpowering systemic energy expenditure and immune surveillance in an evolutionary manner while disease progress. Three approaches were proposed to be investigated, they're: 1). Study of systemic factors, e.g., lipoproteins, promote cancer prognosis; 2). Study of tumor-tropic factor that influence systemic homeostasis, e.g., cancer cachexia; and 3). Study of evolutionary interaction between tumor and host (not yet initiated). During the funding term of NHRI (2018~2024), our team have some progresses in 1st approach of EOLT hypothesis.

1. Hypoxia mediated histone lactylation switch glucose dependent to lipid addicted cancer growth
   a). Hypoxia-Kla related gene cluster positively correlated with cancer prognosis and VLDLR expression.
   b). Hypoxia induced histone lysine lactylation (Kla) turn on lipid entry, Very Low-Density Receptor (VLDLR), expression in HCC.
   c). PG and PE O–/PC O– (VLDLR associated lipids) promotes Kla under hypoxic condition through MGO (Monoglyoxylation) pathway.
   d). Kla-VLDLR expression promote cell growth and mobility.
2. PPARα downregulation compromises lipid scavenging to drive cancer progression:
   a). Lipidome is independent cancer prognostic marker, in spite of transcriptome in patient.
   b). Among prognostic lipids, abundance of ether-linked glycerophospholipids, e.g., PC O– and PE O–, are the dominant lipid classes in association with metastatic and poor prognostic phenotypes.
   c). PC O– and PE O– accumulate via downregulation of PPARα-related lipophage.
   d). PC O– and PE O– promote cell mobility through TRPV.2 related calcium signaling.
3. Lipid overindulgence through Very Low-Density Receptor (VLDLR) promote cancer progression.
   a). Diet-related PC O– and PE O– abundance are prognostic factors in cancer patients.
   b). Lipid importer, VLDLR, upregulated in cancer lesion, of which positive correlated to patient prognosis.
   c). Knocking out VLDLR ablate tumor burden in spontaneous HBVtg-HCC mouse model.
   d). Knocking out VLDLR suppress PE O– and PC O- abundance, by which suppress cell mobility.
   e). Knocking out VLDLR suppress PG abundance. Therefore, disturb membrane fluidity to diminished VEGFR-related signaling.

The cancer metabolic reprograming switches glucose dependence to lipid addiction via hypoxia-Kla-VLDLR axis. The deficit of lipid clearance due to PPARα downregulation compromise lipophagic caliber in cancer cells, which result in ether-lipid accumulation. The prognostic ether-lipids, promotes TRPV.2 calcium dependent signals to facilitate cancer mobility. In summary, our team discovered a lipid metabolic pathophysiology of cancer, echo to EOLT hypothesis.

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Education

2017-2019	Bachelor of Laws, National Tsing Hua University
1998-2004	Ph.D., Department of Basic Medical Sciences, National Cheng Kung University
1993-1997	Bachelor, Department of Biology, National Cheng Kung University, Taiwan

Research and Professional Positions Held in Chronological Sequence

2004-2006	Postdoctoral Fellow, National Health Research Institutes, Taiwan
2006-2009	Postdoctoral Fellow, Max-Planck Institute of Biochemistry, German
2009-2010	Project leader, Biozentrum, University of Basel, Switzerland
2010-2015	Assistant Professor, Institute of Molecular and Cellular Biology, National Tsing Hua University
2015-2021	Associate Professor, Institute of Molecular and Cellular Biology, National Tsing Hua University
2021-present	Professor, Institute of Molecular and Cellular Biology, National Tsing Hua University
2022-present	Professor, School of Medicine, National Tsing Hua University

Research Interests

Dr. Wang is fascinated by the molecular mechanisms of mitotic chromosome segregation in health and disease. Using chronic hepatitis B virus (HBV) as a model, Dr. Wang found that viral oncoproteins, particularly the pre-S mutant large surface antigen, induce ER stress, DNA damage, SOCE activation, and metabolic changes through PKM2 modulation, leading to cytokinesis disruption and hepatocyte hyperploidy (*Hepatology 2001/2005, Molecular Cell 2012, Journal of Pathology 2015/2018, PLOS Pathogens 2021, Antiviral Research 2022/2023*). To understand chromosome segregation control, Dr. Wang studies the interaction between kinetochores and microtubules during chromosome alignment and the spindle assembly checkpoint (*Journal of Cell Science 2010, Molecular Cell 2013, Nature Communications 2015, Journal of Cell Biology 2022, PNAS 2024*). Recently, Dr. Wang has investigated the role of KIF2C in paclitaxel resistance in triple-negative breast cancer (TNBC). Her team demonstrated that KIF2C, a microtubule depolymerase, can mitigate over-stabilized microtubules in the presence of paclitaxel. Supported by NHRI, Dr. Wang's research group has recently developed a potent, cell-permeable KIF2C inhibitor, 7S9 (in revision), which could address chemoresistance to microtubule-targeting agents in the future.

Major Honors and Awards

2023	國立成功大學醫學院校友傑出成就獎
2023	財團法人肝病防治學術基金會 29 週年研究獎助
2022	Elected Full Member of Sigma Xi
2020	國立清華大學 傑出教學獎
2019	財團法人肝病防治學術基金會 25 週年研究獎助
2016	Promising Women in Science Award 吳健雄學術基金會台灣女科學家新秀獎
2014	Publication award, National Tsing Hua University
2013	Publication award, National Tsing Hua University
2008	Max-Planck Gesellschaf ein Forbildungsstipendium
2006	Max-Planck Gesellschaf ein Forbildungsstipendium
2006	財團法人成杏基金會優秀論文獎
2005	財團法人宋瑞樓教授學術基金會優秀論文獎

From Microtubule Depolymerase to Drug Resistance: The Role of KIF2C

Lily Hui-Ching Wang
王慧菁

Institute of Molecular and Cellular Biology, National Tsing Hua University, Taiwan

Kinesins are a large group of eukaryotic-conserved motor proteins that move along microtubules with ATP hydrolysis. Unlike other kinesins, the members of the kinesin-13 family do not use the energy from ATP turnover to move directionally along microtubules, but, instead, depolymerize them by disassembling microtubules from the polymer end. The human genome contains four genes encoding kinesin-13 family members, KIF2A, KIF2B, KIF2C, and KIF24, all of which are required for mitosis but have different roles. Kinesin family member 2C (KIF2C), also known as the mitotic centromere-associated kinesin (MCAK), is located on the centromere and regulates microtubule turnover at the kinetochore. Accordingly, KIF2C is essential for the correction of error-attached spindle microtubules at the kinetochore during chromosome alignment in mitosis.

With the application of systems biology approaches, we identified KIF2C as a distinct biomarker in triple-negative breast cancer (TNBC). Notably, both KIF2C and tubulin polyglutamylation were increased in TNBC cell lines developed with paclitaxel resistance. We demonstrated that KIF2C exhibits a preference for polyglutamylated tubulin as a substrate, persisting even in the presence of paclitaxel, and developed a novel KIF2C inhibitor 7S9, which prohibited the dissociation of KIF2C from microtubules. The combination of 7S9 and paclitaxel showed synergistic effect in cytotoxicity in chemoresistant cells and also significantly suppressed tumorigenesis of chemoresistant TNBC *in vivo*. Interestingly, we found that paclitaxel-resistant cells developed cross-resistance against the majority of clinically-available microtubule-targeting agents (MTAs). We show that the inhibition of KIF2C by 7S9 could overcome the chemoresistance against all these MTAs. These findings shed light on the molecular mechanism of KIF2C-mediated chemoresistance, emphasizing KIF2C as a crucial cross-resistance target in TNBC (manuscript in revision).

In addition to MTAs, we recently found that inhibition of KIF2C could synergistically enhance cytotoxicity mediated by a group of anti-cancer drugs that cause DNA damages. In light with a recent report that cytoplasmic microtubules may participate DNA double strand break (DSB) repositioning during damage repair (Nature Structural & Molecular Biology, 2024, doi.org/10.1038/s41594-024-01286-7), we propose that KIF2C may have a non-canonical function on DNA repair through modulating the DSB–capturing nuclear envelope tubules. Detailed mechanisms will be elucidated in future studies.

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Education

1990-1995	B.S., Veterinary Medicine, National Taiwan University, Taiwan
1995-1997	M.Sc., Biology, University of Waterloo, Ontario, Canada
1997-2002	Ph.D., Medical Sciences, McMaster University, Ontario, Canada

Research and Professional Positions Held in Chronological Sequence

2002-2005	**Post-doctoral Fellow,** Department of Biological Science, University of Calgary, Calgary, Alberta, Canada
2005-2011	**Assistant Professor**, Graduate Institute of Physiology, National Taiwan University College of Medicine, Taipei, Taiwan
2011-2016	**Associate Professor**, Graduate Institute of Physiology, National Taiwan, University College of Medicine, Taipei, Taiwan
2016-present	**Professor**, Graduate Institute of Physiology, National Taiwan University College of Medicine, Taipei, Taiwan

Research Interests

Our laboratory investigates cellular and molecular mechanisms of intestinal pathophysiology and mucosal immuno-oncology. **Our research focuses on host-microbe interaction for the regulation of epithelial barrier and tumorigenesis in the gastrointestinal tract.** Epithelial dysfunction and microbiota dysbiosis play crucial roles in the pathogenesis of various gastrointestinal diseases, such as inflammatory bowel disease and colorectal cancer. We are particularly interested in the control of epithelial transcellular and paracellular barriers and their relationship with microbiota shaping in chronic disorders, and aim to delineate how dysregulated epithelial innate signaling contributes to disease progression. One of the major focuses of our work is to understand the alteration of microbiota diversity and the emergence of commensal-derived pathobionts during inflammation and cancer initiation. We isolated invasive pathobionts from colonic epithelial cells for whole genome sequencing to decipher the distinction between commensals and pathobionts in the bacteriome community. **Our laboratory further explores intestinal virome and the existence of bacteriophages at lytic and lysogenic cycles in the microbiota by using a metagenomics approach which helps fill the knowledge gap on viral dark matter and address the impact of bacteria-virus community networks in disease pathogenesis.** Our findings provided insights into the key time frame and core mechanistic interplay between host and microbes for driving inflammation and carcinogenesis. The long-term objective is to develop novel therapeutics directed at restoring epithelial homeostasis and correcting microbiota ecosystem to prevent inflammatory flare and curb cancer progression.

Major Honors and Awards

2002-2005	Canadian Association of Gastroenterology (CAG)/AstraZeneca Research Initiative Award, Postdoctoral Fellowship
2003	Young Investigator Prize, Banff Inflammation Workshop 2003, Alberta, Canada
2004	Mucosal Inflammation Research Group Trainee Travel Prize, University of Calgary, Nitric oxide, Cytokines, and Inflammation International Symposium, Rio de Janeiro, Brazil
2005	Banff Inflammation Workshop Trainee Bursary and Best Poster Presentation Award, University of Calgary, Alberta, Canada
2009	Young Investigator Award, Asia Pacific Digestive Week (APDW) 2009
2011	Young Investigator Award, The 7th Federation of the Asian and Oceanian Physiological Societies (FAOPS) Congress 2011
2016	Fellowship of American Gastroenterology Association (AGAF), USA
2013-2016	Outstanding Young Investigator Project Grant, Principle Investigator, Ministry of Science and Technology, Taiwan.
2017, 2018, 2019	Taiwan SPARK Awardee, Candidate team, Biotechnology Innovation Organization (BIO) Convention,
2019	The 16th National Innovative Award, Academic Research Innovation Group, Pharmacological development and Precision Medicine. Research Center for Biotechnology and Medicine Policy (RBMP), Taiwan
2016-2026	Council member, the 24th, 25th, 26th, 27th, 28th Council Board of the Chinese Physiological Society (CPS), Taiwan
2020-2026	Director of International Affairs and Physiology Education Committee (IAPEC), Chinese Physiological Society (CPS), Taiwan
2019-2023	Council member of Federation of Asian and Oceanian Physiological Societies (FAOPS)
2023-2027	Treasurer of Federation of Asian and Oceanian Physiological Societies (FAOPS)
2022-2025	Council member and Chair of Commission V: secretion and absorption, International Union of Physiological Sciences (IUPS)
2023	Future Tech Award (FUTEX), National Science and Technology Council (NSTC). Category: Biotechnology and Medical Devices.
2023	The 19th National Innovative Award, Academic Research Innovation Group, Pharmacological development and Precision Medicine. Research Center for Biotechnology and Medicine Policy (RBMP), Taiwan

Host-microbiota Interaction in Intestinal Tumorigenesis: Three-way Crosstalks between Epithelia, Bacteriome, and Virome

Linda Chia-Hui Yu
余佳慧

Graduate Institute of Physiology, National Taiwan University, Taiwan

A massive amount of microorganisms, such as bacteria, viruses, archaea, and fungi, inhabits the human gastrointestinal tract. Segregation of the host and microbiota by epithelial barriers is crucial for the maintenance of a symbiont relationship in a health status. Aberrant host-microbiota interaction contributes to disease development. Recent evidence from our laboratory indicates that microbiota dysbiosis and emergence of invasive pathobionts (commensal-derived opportunistic pathogens) play causative roles in chronic inflammation and colitis-associated colorectal cancers. An invasive *Escherichia coli* strain isolated from colonocytes acted as tumorigenic pathobionts that involved suppression of cellular autophagy and adversely caused free radical overproduction, leading to epithelial hyperproliferation and cancer initiation. The invasive *E. coli* also upregulated cancer stemness markers and increased tumorsphere formation, implicating multiple pathways for bacteria-associated tumor growth. Despite a clear link between bacteriome and tumorigenesis, the importance of virome remains mostly neglected. Although significant advances are made in high-throughput sequencing, the majority of datasets are dominated by uncharacterized sequences unmatched to any databases, termed 'viral dark matter'. By keeping this in mind, fecal virome is primarily composed of bacteriophages (a virus that infects bacterial host). Our gene microarray data revealed abnormal antiviral interferon responses in mouse colonic tissues, suggesting a role of virus in tumor development. From the microbial side, unique genetic signatures and lysogenic prophage sequences with complete viral structural elements were identified in the chromosome of the invasive *E. coli*. Packaging and release of prophage-derived viral particles from the *E. coli* was noted upon oxidative stress. In addition, treatment with a newly identified lytic phage exerted tumor reduction in a preclinical mouse model. By using a metagenomic approach, we hope to address the knowledge gap of cancer virome and decipher how bacteria-phage community networks impact tumorigenesis. The understanding of microbiome-CRC etiology will benefit the development of novel microbe-based precision therapy for cancer patients.

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Education

1983-1987	B.S., Department of Plant Pathology & Microbiology, National Taiwan University, Taipei, Taiwan
1987-1991	Ph.D., Institute of Biochemistry & Molecular Biology, National Taiwan University, Taipei, Taiwan

Research and Professional Positions Held in Chronological Sequence

1991-1994	Visiting Postdoctoral Biologist at the Department of Biology, University of California, San Diego, USA.
1994-1995	Postdoctoral Researcher at the Cancer Clinical Research Center Laboratory, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
1995-1997	Senior Postdoctoral Researcher at the Division of Cancer Research, National Health Research Institutes, Taipei, Taiwan
1997-2002	Assistant Investigator at the Division of Cancer Research, National Health Research Institutes, Taipei, Taiwan
2002-2014	Associate Investigator at the National Institute of Cancer Research, National Health Research Institutes, Miaoli, Taiwan
2014-present	Investigator at the National Institute of Cancer Research, National Health Research Institutes, Miaoli, Taiwan

Research Interests

Solid tumor is not only composed of neoplastic epithelial cells, rather, it comprises a range of proliferating stromal cells such as fibroblasts and macrophages. Dr. Huang's research team has been focusing on the tissue microenvironment reprogramming for cancer development and progression. They have exploited several transgenic and mixed cells-transplanted mouse models to explore the cell-cell interplays in the desmoplastic tumor microenvironment. Desmoplasia is a common hallmark of many malignancies including non-small cell lung adenocarcinoma, hepatocellular carcinoma, colorectal cancer, and pancreatic ductal adenocarcinoma (PDAC). In the PDAC tumor, desmoplastic stroma generally constitutes 70~90% of total tumor volume and is closely associated with rapid tumor growth, immunosuppression, metastasis, and therapeutic resistance. Dr. Huang's laboratory pays much attention on the development of novel agents/strategies for treatment of tumor desmoplasia. Considering cachexia is the leading cause of death in PDAC patients, Dr. Huang's laboratory is also interested in the inter-organ communications, especially between the PDAC tumor and muscle/adipocytic tissues.

Major Honors and Awards

2022	19th National Innovation Award, Taiwan
2023	National Innovation Excelsior Award, Taiwan

Targeting Extracellular HSP90α as Novel Anti-Desmoplasia and Anti-Cachexia Strategy for Pancreatic Adenocarcinoma Therapy

Chi-Shuan Fan[1], Hui-Chen Hung[2], Li-Li Chen[1], Chia-Chi Chen[1], Yi-Yu Ke[2], Teng-Kuang Yeh[2], Teng-Yuan Chang[2], Kuei-Jung Yen[2], Chin-Ting Huang[2], Chung-Hsing Chen[1], Kee Voon Chua[1], Zhao-Lin Tan[1], John Tsu-An Hsu[2], Tze-Sing Huang[1]
范吉炫[1], 洪慧貞[2], 陳麗莉[1], 陳佳琪[1], 柯屹又[2], 葉燈光[2], 張騰元[2], 嚴貴榮[2], 黃靖婷[2], 陳中興[1], 蔡綺雯[1], 譚兆麟[1], 徐祖安[2], 黃智興[1]

[1]National Institute of Cancer Research, National Health Research Institutes, Taiwan
[2]Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Taiwan

Desmoplasia commonly occurs in many malignancies including pancreatic ductal adenocarcinoma (PDAC). The increased mesenchymal cells arise not only from the proliferation of tissue-resident fibroblasts, but also from the mesenchymal transition of other cell types maintaining cell plasticity. Extracellular HSP90α (eHSP90α) has been identified as a key facilitator of cellular mesenchymal transition and activation. In this study, the involvement of eHSP90α in tumor desmoplasia was validated by a tumor transplant mouse model. The inclusion of endothelial-mesenchymal transition cells into PDAC cell grafts significantly promoted desmoplastic tumorigenesis with elevated serum eHSP90α levels, which was drastically prevented in mice treated with anti-HSP90α antibody. Cell culture studies highlighted eHSP90α's induction of TCF12-mediated mesenchymal transition and production of collagen Iα1 and hyaluronic acid synthases in macrophages and epithelial and endothelial cells. Therefore, we developed and evaluated a humanized anti-HSP90α antibody HH01 as a novel anti-desmoplasia agent. HH01 exhibited a high affinity for eHSP90α and effectively blocked eHSP90α's binding with the cell-surface receptor CD91, thereby preventing downstream tumor-promoting effects and self-induction of excess eHSP90α. In desmoplastic tumor transplant mouse models, HH01 alone drastically abrogated desmoplasia and tumor growth, and exhibited a synergistic effect with gemcitabine. In pancreatic K-Ras^{G12D} transgenic mice, HH01 suppressed pancreatic desmoplasia, PDAC development, liver metastasis, and notably extended survival. HH01 treatment also modulated tumor immunity, reducing M2-macrophages and reinvigorating immune T cells. Given cachexia is the leading cause of death in PDAC patients, we observed that tumor size was not the key factor leading to cachexia; rather, it is tumor desmoplasia and the associated high levels of eHSP90α that are related to the onset of cachexia. HH01 therapy also exhibited efficacy to repress the desmoplasia-associated cachexia. Taken together, our results suggest a promising therapeutic strategy against the desmoplasia and cachexia challenges of PDAC.

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Education

1983-1987	B.S., Electrical Engineering, National Taiwan University, Taiwan
1989-1990	M.S., EE: Systems, University of Michigan, U.S.A.
1991-1994	Ph.D., EE: Systems, University of Michigan, U.S.A.

Research and Professional Positions Held in Chronological Sequence

1990-1994	Research Assistant, Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI 48109-2122, U.S.A.
1994-1997	Member of Technical Staff (System Design Engineer), Imaging Technologies, Sequoia Engineering, Acuson Corporation, Mountain View, CA 94039-7393, U.S.A.
1997-1998	Assistant Professor, Electrical Engineering, National Taiwan University, Taipei, Taiwan
1998-2003	Associate Professor, Electrical Engineering, National Taiwan University, Taiwan
2001-2004	Adjunct Associate Investigator, Division of Medical Engineering Research, National Health Research Institutes, Taiwan
2003-present	Professor, Electrical Engineering, National Taiwan University, Taiwan
2004-present	Adjunct Investigator, Division of Medical Engineering Research, National Health Research Institutes, Taiwan
2006-2009	Founding Director, Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taiwan
2008-present	Distinguished Professor, National Taiwan University, Taiwan
2009	Founding Director, Yong-Lin Biomedical Engineering Center, National Taiwan University, Taiwan
2015-2018	Associate Dean, College of Electrical Engineering and Computer Science, National Taiwan University
2016-2018	Convener, Biomedical Engineering Program, Ministry of Science and Technology, Taiwan
2019-2023	Vice President for Research and Development, National Taiwan University Director, Industry Liaison Office, National Taiwan University
2019-present	Member, Advanced Research Advisory Committee (ARAC), Industrial Technology Research Institute (ITRI) , Taiwan

Research Interests

Biomedical Engineering, Biomedical Photoacoustics, Ultrasound Imaging and Therapy, Medical Device Innovation

Major Honors and Awards

1992	The 10-th Annual National VLSI Contest Award
1994	Distinguished Achievement Award, University of Michigan
1998-2001	Research Award, National Science Council, Taiwan, R.O.C.
2002	-Dr. Wu Dayou Research Award, National Science Council -Outstanding Young Electrical Engineer Award, Chinese Institute of Electrical Engineering -Distinguished Industrial Collaboration Award, Ministry of Education
2003	Outstanding Researcher Award, National Taiwan University
2004	-Distinguished Research Award, National Science Council -Distinguished Research Achievement Award, National Taiwan University
2005	Outstanding Electrical Engineer Professor Award, Chinese Institute of Electrical Engineering
2008	IEEE Fellow
2009	-IAMBE Fellow -Distinguished Research Award, National Science Council
2011	-NTU Distinguished Innovation Research Reward, National Taiwan University -National Innovation Award
2012	-Distinguished Research Award, National Science Council, -AIUM Fellow
2014	TBF (Taiwan Bio-development Foundation) Chair in Biotechnology
2015	SPIE Fellow
2016	Getac Chair
2017	Y. Z. Hsu Science Award
2018	-IFMBE Vladimir K. Zworykin Award -Outstanding Engineering Professors Award, Chinese Institute of Engineers
2019-2020	IEEE UFFC Distinguished Lecturer(2019/1-2020/6)
2019	Academia Award, Ministry of Education
2019	AmTRAN Chair
2022	Merit MOST Research Fellow Award, Ministry of Science and Technology
2022	IFMBE Otto Schmitt Award
2024	AIUM Joseph H. Holmes Basic Science Pioneer Award

Elasticity Measurements of 3D Cell Culture Systems:
Principles, Devices, and Potential Applications to Stiffness-Targeted Treatment Delivery

Pai-Chi Li
李百祺

Department of Electrical Engineering, National Taiwan University, Taiwan

Recent studies on drug screening and disease progression have shown that 3D cell culture systems can better represent the *in vivo* conditions than 2D monolayer cultures. Studying mechanobiology in 3D cell culture systems also recapitulates cell behaviors in response to various types of mechanical stimuli. An effective tool for measuring the spatiotemporal changes in elastic properties of such 3D cell culture systems without invasively contacting the samples has not been readily available but is undoubtedly needed. We have developed novel optical and acoustic shear wave imaging methods and devices for non-invasive quantification of the matrix stiffness in 3D culture conditions. Such methods require both high sensitivity and adequate spatial resolution. In particular, the complementary physical properties of light and sound are exploited, and innovative devices are developed, even though these two distinctly different physical mechanisms are often separately applied to biomedical problems. Several techniques were developed, and their applications were demonstrated. In addition, we will present a simple setup for shear wave elasticity imaging using only one single element transducer with machine learning based image reconstruction. The applications of the proposed techniques in *in vitro* research for radiation therapy will also be presented. Our experiment results demonstrate the significant influence of extracellular matrix (ECM) stiffness on liver cancer's response to radiation. On the other hand, sonoporation-aided lysyl oxidase (LOX) inhibition emerges as a promising strategy to mitigate stiffness-related resistance, offering potential improvements in liver cancer treatment outcomes.

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Education

1998~2002	B.S., Biomedical Engineering, Chung Yuan Christian University, Taiwan
2002~2003	M.S., Biomedical Engineering, Chung Yuan Christian University, Taiwan
2003~2007	Ph.D., Biomedical Engineering, Chung Yuan Christian University, Taiwan

Research and Professional Positions Held in Chronological Sequence

2008-2012	Assistant Professor, Department of Electrical Engineering, Fu Jen Catholic University, Taiwan
2012~2013	Assistant Professor, Department of Biomedical Engineering, National Cheng Kung University, Taiwan
2013~2015	Assistant Professor, Department of Biomedical Engineering, National Cheng Kung University, Taiwan
2015~2018	Associate Professor, Department of Biomedical Engineering, National Cheng Kung University, Taiwan
2018~2023	Professor, Department of Biomedical Engineering, National Cheng Kung University, Taiwan
2021~2022	Visiting Professor, Department of MAE, North Carolina State University, USA
2023~present	Distinguished Professor, Department of Biomedical Engineering, National Cheng Kung University, Taiwan

Research Interests

High frequency ultrasound imaging, Ultrafast ultrasound imaging, Super-resolution blood flow imaging, High-resolution shear wave elastography, Sonodynamic therapy, Doppler flowmeter design, Ultrasonic tissue characterization, Hemodynamic research, Biomedical electronic equipment design.

Major Honors and Awards

2009	Outstanding research Award, Fu Jen Catholic University
2011	Outstanding research Award, Fu Jen Catholic University
2012	The Best Advisor Award, Creative Design and Implementation Competition on Biomedical Engineering, Taiwan
2012	JMBE Annual Excellent Paper Award, Taiwan
2014	Best Potentiality Award, Biomedical Engineering Innovation Competition, Taiwan
2016	Rising Star Award, College of Engineering, National Cheng Kung University, Taiwan

2017	The Best Annual Paper Award, Journal of Medical and Biological Engineering (SCI)
2020	Ministry of Science and Technology Future Tech Award, Taiwan
2021	Excellent Research Award, Engineering College, National Cheng Kung University, Taiwan
2022	Ministry of Science and Technology Future Tech Award, Taiwan
2023	Ministry of Science and Technology Future Tech Award, Taiwan
2023	IEEE Tainan Section Outstanding Technical Achievement Award

Ultrafast High Frequency Ultrasound Imaging and Its Biomedical Applications

Chih-Chung Huang
黃執中

National Cheng Kung University, Department of Biomedical Engineering, Taiwan

The frame rate (the number of images displayed in one second) of current ultrasound imaging machines is about 30-100 fps, which is sufficient for most clinical applications, even echocardiography. However, high frame rate ultrasound imaging, so-called ultrafast ultrasound imaging, has recently been achieved with improvements of ultrasound hardware. One major advantage of ultrafast ultrasound imaging is that it converts the ultrasound image into a “high speed camera”, in which any movement of an object in the view of ultrasound exhibits “slow motion”. Currently, use of plane wave imaging is the standard for ultrafast ultrasound imaging, which the frame rate of ultrafast imaging can be up to >10 kHz. With the emergence of ultrafast ultrasound imaging, several new applications of ultrasound imaging have been proposed such as shear wave elastography, super resolution blood flow imaging, and ultrasound contrast imaging.

To date, the operational frequency of the ultrafast ultrasound imaging is around 2 to 18 MHz, which provides sufficient image resolution with an appropriate imaging depth for clinical applications. However, the spatial resolution of ultrasound imaging can be improved by using high frequency ultrasound imaging (HFUS, >30 MHz), for example, 50 MHz ultrasound imaging provideslateralandaxialresolutionsof100and20 μm, respectively. Recently, single element and array transducers HFUS imaging systems are commercially available at a center frequency up to 50 MHz. However, the HFUS with an ultrafast imaging ability is still lacking currently.

In this talk, ultrafast HFUS imaging combining a high frequency array transducer (~40 MHz) with a programmable ultrasound imaging has been proposed. Due to the high imaging resolution ability of HFUS, this ultrafast HFUS imaging is suitable for superficial tissue imaging of human and small animal for gene research and cancer studies. Therefore, high-resolution ultrasound elastography and super resolution blood flow imaging without micro-bubble are currently available for biomedical applications, such as high resolution elastography for human cornea, skin, hand tendon, and mouse brain as well as super resolution blood flow imaging for small animal applications in nerve, brain, heart, and kidney.

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Education

1987-1994	Bachelor, Medicine, Kaohsiung Medical University, Taiwan
2004-2013	Ph.D., Graduate Institute of Medicine, Kaohsiung Medical University, Taiwan

Research and Professional Positions Held in Chronological Sequence

2003-	Attending Physician, Department of Orthopaedic Surgery, Kaohsiung Medical University Hospital, Taiwan
2008-2012	Assistant Professor, Kaohsiung Medical University
2012-2014	General Secretary, Taiwan Orthopedic Research Society
2012-2019	Associate Professor, Kaohsiung Medical University
2016-2018	Deputy General Secretary, Taiwan Orthopedic Association
2017-2018	General Secretary, Pacific and Asian Society of the Minimally Invasive Spine Surgery
2017-2019	General Secretary, Taiwan Osteoporosis Association
2019-	Professor, Kaohsiung Medical University
2019-	Director, Orthopaedic Research Center, Kaohsiung Medical University
2020-2022	President, Taiwan Orthopedic Research Society
2023-2025	President, The Taiwanese Osteoporosis Association

Research Interests

Osteoarthritis
Osteoporosis
Osteonecrosis
Intervertebral disc development and degeneration
Chondral defect
Bone defect
Tissue engineering

Major Honors and Awards

2012	The 8th national Innovation award: Treatment of early-stage arthritis
2013	2nd Prize of High Impact Factor Paper Award, Taiwan Orthopedic Research Society
2013	1st Prize of Best Paper Award in Clinical Science, Taiwan Orthopedic Association
2013	1st Prize of Best Paper Award in Basic Science, Taiwan Orthopedic Association

2014	Merit Award (Best paper session), 2nd AO Trauma Asia Pacific Scientific Congress& TK Expert's Symposium
2015	1st Prize of Best Paper Award, Taiwan Orthopedic Research Society
2016	1st Prize of Best Paper Award, Taiwanese Osteoporosis Association
2017	1st Prize of Best Paper Award, Taiwanese Osteoporosis Association
2018	1st Prize of Award of the best abstract, The Acelity Surgical Wound Forum
2023	Top 2% Scientist
2024	Best Paper Award, Joint Reconstruction Society, ROC

Parathyroid Hormone (PTH) for Osteoarthritis Treatment: from Bench to Pre-clinical

Chung-Hwan Chen, Je-Ken Chang, Mei-Ling Ho
陳崇桓，張瑞根，何美泠

Department of Orthopaedic Surgery, Kaohsiung Medical University, Taiwan

Osteoarthritis (OA) is a crucial disease in the field of orthopedics and is the most prevalent joint disease worldwide. The main pathogenesis of OA is characterized by phenotypic changes in the articular chondrocytes in which they experience terminal differentiation and eventually undergo apoptosis in growth plate cartilage. Parathyroid hormone (PTH) is the most important regulator of calcium homeostasis through its direct action on the bones and kidneys. PTH-related protein (PTHrP) maintains chondrocyte proliferation and inhibits the chondrocyte differentiation that leads to hypertrophy. PTH analogs and PTHrP both act through the type I PTH receptor to regulate chondrocyte differentiation. We found that PTH increased the mRNA expression of type II collagen (Col II) and reduced the mRNA expression of type X collagen (Col X) in chondrocytes *in vitro*. We also determined that that a low dose of intra-articular PTH (1-34) prevented chondrocyte apoptosis and increased the Safranin O–stained area in rats with papain-induced OA. Moreover, PTH (1-34) ameliorated knee OA through autophagy after anterior cruciate ligament transection (ACLT) and reduced dexamethasone-induced terminal differentiation in human articular chondrocytes. In addition, we demonstrated that intra-articular PTH (1-34) injections improved spontaneous OA by directly affecting the cartilage rather than the subchondral or metaphyseal bone in a preclinical age-related OA model. In the function test, PTH (1-34) can prolong the time on treadmill test and increase the ratio of weight bearing. In the large animal study, PTH (1-34) also can improve osteoarthritis in goat receiving partial menisectomy by ameliorate GAG loss, histological score and improve weight bearing ratio in goat. Therefore, PTH may be a good candidate for OA treatment research.

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Education

1997-2001	B.S., Electrical Engineering, National Taiwan University, Taipei, Taiwan
2001-2003	M.S., Electrical Engineering, National Taiwan University, Taipei, Taiwan
2003-2008	Ph.D., Electrical Engineering, National Taiwan University, Taipei, Taiwan

Research and Professional Positions Held in Chronological Sequence

2009-2010	Postdoctoral Research Fellow, Center for Optoelectronic Biomedicine, National Taiwan University College of Medicine, Taipei, Taiwan
2010-2011	Visiting Fellow, Advanced MRI Section, Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA
2011-2013	Assistant Investigator, Division of Medical Engineering Research, National Health Research Institutes, Miaoli, Taiwan
2013-2019	Assistant Investigator, Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Miaoli, Taiwan
2016-2023	Adjunct Assistant Professor, Institute of Medical Device and Imaging, National Taiwan University College of Medicine, Taipei, Taiwan
2019-Present	Associate Investigator, Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Miaoli, Taiwan
2020-2021	Jointly Appointed Assistant Professor, Institute of Biomedical Engineering, National Chiao Tung University, Hsinchu, Taiwan
2021-2022	Jointly Appointed Associate Professor, Institute of Biomedical Engineering, National Chiao Tung University, Hsinchu, Taiwan
2023-Present	Jointly Appointed Associate Professor, Institute of Biomedical Engineering, National Yang Ming Chiao Tung University, Hsinchu, Taiwan
2023-Present	Adjunct Associate Professor, Institute of Medical Device and Imaging, National Taiwan University College of Medicine, Taipei, Taiwan

Research Interests

During the past few years, our research has focused on developing the MRI core technology platform, novel MRI systems, MRI methodology, neuroimaging methods, and facilitating their use on a variety of pre-clinical and translational applications. For MRI core technology development, we continue to strengthening the capacity of the multi-scale connectome MRI system platform with ultra-high gradients we developed at NHRI, including novel radio-frequency coil design, MRI-guided focused

ultrasound platform, MRI ghost removal algorithm, and motion correction. For technical development of MRI methodology and neuroimaging methods, we have developed novel diffusion MRI techniques to map the brain connectomics and tissue microstructures. For research on translational applications, we aimed to decipher the complex brain networks in health and disease states, and innovate data acquisition, analysis methods and imaging biomarkers to improve the data quality and diagnostic accuracy in clinical applications. Specifically, we have developed novel MR neuroimaging methods to investigate the structural and functional alterations and explore their association with psychological evaluation in neurodegenerative diseases by collaborating closely with clinician and psychologists. Recently, we have great interests in exploring the human congential brain malformation and fetal brain development. A dedicated and unique MRI platfrom has been established at NHRI for imaging the postmortem brains with high spatial resolution and multi-parametric contrasts. This platform is essential to understand the human brain development and its underlying mechanism.

Major Honors and Awards

2010-2011	Postdoctoral Research Abroad Fellowship, National Science Council, Taiwan
2010-2011	Visiting Fellowship, Intramural Research, National Institute of Neurological Disorders and Stroke, National Institutes of Health, USA
2014	National Invention and Creation Award, Taipei, Taiwan
2020	NHRI Research Achievement Award for Young Scientist, NHRI, Taiwan
2020	National Innovation Award, Taipei, Taiwan
2021	The ISMRM Summa Cum Laude Merit Award, 2021 ISMRM Annual Meeting
2023	Gold Medal Award, 2023 Taiwan Innotech Expo Invention Contest, Taipei, Taiwan

Deciphering Tissue Microstructures and Neural Connectivity of Postmortem Fetal Brains with High-resolution Connectome MRI

Sheng-Min Huang[1], Kuan-Hung Cho[2], Koping Chang[3,4], Pei-Hsin Huang[3,4], Li-Wei Kuo[1]
黃聖閔[1], 卓冠宏[2], 張克平[3,4], 黃佩欣[3,4], 郭立威[1]

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[2]Department of Electronic Engineering, National United University, Taiwan;
[3]Department of Pathology, National Taiwan University Hospital, Taiwan;
[4]Graduate Institute of Pathology, National Taiwan University College of Medicine, Taiwan.

As human brain coordinates cognition, learning, memory, and social behaviors, the development and maturation of brain lasting from fetus to early childhood possess the major role in human growth. Abnormalities in brain structures during development often lead to impairments in language, intelligence, emotion, as well as neurological/psychiatric disorders. In order to gain more insights into the development of fetal brains, we have developed a dedicated multi-contrast, high-resolution *ex vivo* brain MRI platform at NHRI and aimed to map the tissue microstructures and neural architectures of postmortem human fetal brains. Novel strategy of simultaneous whole brain slicing was also innovated and successfully performed on extremely soft human fetal brains. In this study, we have focused on the lissencephaly, also known as smooth brain, which is a congenital brain malformation characterized by absence of gyri and sulci caused by defective cortical growth and folding. Specifically, thalamocortical (TC) fiber growth begins during the embryonic period and completes by the third trimester of gestation, so that human neonates at birth have a thalamus with a near-facsimile of adult functional parcellation. Whether congenital neocortical anomaly (e.g., lissencephaly) affects TC connection in humans is unknown. Therefore, via diffusion MRI fiber-tractography analysis of long-term formalin-fixed postmortem fetal brain diagnosed as lissencephaly in comparison with an age-matched normal one, we found similar topological patterns of thalamic subregions and of internal capsule parcellated by TC fibers. However, lissencephaly fetal brain showed white matter structural changes, including fewer/less organized TC fibers and optic radiations, and much less cortical plate invasion by TC fibers — particularly around the shallow central sulcus. Diffusion MRI fiber tractography of normal fetal brains at 15, 23, and 26 gestational weeks (GW) revealed dynamic volumetric change of each parcellated thalamic subregion, suggesting coupled developmental progress of the thalamus with the corresponding cortex. Our study thus shows the feasibility of connectome MRI in postmortem long-term formalin-fixed fetal brains to disclose the developmental progress of TC tracts coordinating with thalamic and neocortical growth both in normal and lissencephaly fetal brains at mid-gestational stage. Most importantly, the developed multi-contrast, high-resolution *ex vivo* brain MRI platform could facilitate the understanding of human fetal brain development and malformation in congenital disorders.

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Education

1992-1999	M.D., School of Medicine, National Taiwan University, Taiwan
2002-2004	M.S., Graduate Institute of Clinical Medicine, National Taiwan University, Taiwan
2004-2010	Ph.D., Graduate Institute of Clinical Medicine, National Taiwan University, Taiwan
2009-2011	LL.M., Graduate Institute of Clinical Medicine, National Taiwan University, Taiwan
2012-2013	Research fellow, Massachusetts Eye and Ear Infirmary, Harvard Medical School, USA
2011-2020	Ph.D., Graduate Institute of Law, National Taiwan University College of Law, Taiwan

Research and Professional Positions Held in Chronological Sequence

2004-2008	Lecturer, Department of Otolaryngology – Head and Neck Surgery, National Taiwan University College of Medicine, Taiwan
2008-2014	Assistant Professor, Department of Otolaryngology – Head and Neck Surgery, National Taiwan University College of Medicine, Taiwan
2014-2019	Associate Professor, Department of Otolaryngology – Head and Neck Surgery, National Taiwan University College of Medicine, Taiwan
2019-current	Professor, Department of Otolaryngology – Head and Neck Surgery, National Taiwan University College of Medicine, Taiwan
2019-current	Adjunct Professor, Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taiwan
2020-current	Director, Department of Medical Research, National Taiwan University Hospital Hsin-Chu Branch, Taiwan
2020-current	Director, e-Health and Law Research Center, International Artificial Intelligence and Law Research Foundation, Taiwan
2021-current	Director, Department of Otolaryngology – Head and Neck Surgery, National Taiwan University Hospital Hsin-Chu Branch, Taiwan

Research Interests

Dr. Chen-Chi Wu is an attending physician at the Department of Otolaryngology, National Taiwan University Hospital (NTUH) and a professor at the National Taiwan University College of Medicine

(NTUCM). He is currently the director of the Cochlear Implantation Program at NTUH and the director of the Department of Medical Research and the Department of Otolaryngology - Head and Neck Surgery, National Taiwan University Hospital Hsin-Chu Branch.

Dr. Wu's clinical interest lies in pediatric otology, cochlear implantation, endoscopic ear surgery, and lateral skull base surgery. His research interest centers on genetics of deafness, cochlear implantation, minimally-invasive ear surgery, and molecular diagnostics/therapeutics of inner ear diseases. His team has been dedicated to the early detection, diagnosis, and management of childhood hearing impairment over the past 15 years. His previous and current work includes clinical genetic studies, translational studies, and basic animal studies, covering a wide but continuous range of research interests in this field.

Dr. Wu received a M.D. degree from the NTUCM in 1999. After his residency training at the Department of Otolaryngology, NTUH, he attended the Graduate Institute of Clinical Medicine, NTUCM where he graduated with master degree in 2004 and PhD degrees in 2010. From 2012 to 2013, he was trained as a post-doctoral research fellow under the supervision of Prof. Konstantina Stankovic in molecular genetics of deafness at Massachusetts Eye and Ear Infirmary, Harvard Medical School.

Dr. Wu has been extensively involved in numerous international academic societies. He is currently a board member of the Asia Pacific Symposium on Cochlear Implant and Related Sciences (APSCI), and an active member of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNSF), the Association for Research in Otolaryngology (ARO), the American Society of Human Genetics (ASHG), the Barany Society, the International Otopathology Society, and the Politzer Society. Dr. Wu was approved as a member of the prestigious Collegium Oto-Rhino-Laryngologicum Amicitiae Sacrum (CORLAS) in 2022.

Major Honors and Awards

2004-	Member, Phi Tau Phi Scholastic Honor Society
2010	Ching-Xing Medical Award, National Taiwan University
2019	Mr. Lu Feng-Zhang Memorial Medal
2019-	Board member, Asia Pacific Symposium on Cochlear Implant and Related Sciences (APSCI)
2022	Gold medal, Asia-Pacific Sustainability Action Award, APSAA
2022	Kyorin award, Taipei Medical Association
2022-	Member, Collegium Oto-Rhino-Laryngologicum Amicitiae Sacrum (CORLAS)
2022	National Innovation Award
2023	National Innovation Award

Precision Health in Childhood Hearing Loss: from Diagnostics, Prognostics to Therapeutics

Chen-Chi Wu
吳振吉

Department of Otolaryngology, National Taiwan University College of Medicine, Taiwan

Accurate diagnosis and prognosis are critical to improving outcomes for children with hearing loss. As an etiologically heterogeneous condition caused by a variety of genetic and/or environmental factors, the outcome of childhood hearing loss is highly variable. Previous studies, including ours, have identified several prognostic factors (including genetic diagnosis, audiological results, and imaging findings) that may influence the outcome of treatment for childhood hearing loss. Unfortunately, there is still no reliable outcome prediction tool for clinical use, probably due to the heterogeneous etiologies and the lack of integrated clinical data. Meanwhile, although most children with severe hearing loss have good speech perception after cochlear implantation, they do not regain "natural hearing". New biological therapeutic approaches based on gene transfer and gene editing tools are being developed to address these unmet clinical needs.

Recent advances in sequencing technology, artificial intelligence, and molecular therapy have revolutionized clinical approaches and research methodologies for genetic disorders. Over the past decades, we have established a large genomic database of childhood hearing loss in Taiwan, laying the foundation for machine learning-based multiomics studies. Meanwhile, we have also established platforms to study the pathogenetic mechanisms and molecular therapeutics in transgenic mouse models. In this talk, I will share our experiences on how we are using novel tools in sequencing technology, artificial intelligence, and molecular therapy to achieve precision health for childhood hearing loss.

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Education

1988-1992	B.S., Department of Computer Science, Soochow University, Taiwan
1992-1998	Ph.D., Institute of Computer and Information Science, National Chiao Tung University, Taiwan (Ph.D. Thesis: "Compression, Encryption, and Hiding of Still Images")

Research and Professional Positions Held in Chronological Sequence

1998-2003	Postdoctoral Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan (military service)
2003-2007	Assistant Research Fellow, Genomics Research Center, Academia Sinica
2007-2014	Associate Research Fellow, Genomics Research Center, Academia Sinica
2022-2023	Division Director of Physical & Computational Genomics, Genomics Research Center, Academia Sinica
2014-presnt	Research Fellow, Genomics Research Center, Academia Sinica
~present	Joint Professor, NTU-Academia Sinica Genomics and Systems Biology Degree Program, National Taiwan University
~present	Joint Professor, Taiwan International Graduate Program for Biodiversity, National Taiwan Normal University

Research Interests

This is the Information and Systems Biology (ISB) laboratory.

Subject: Comparative and Evolutionary Genomics/Transcriptomics, Causative Biology, Systems Biology, Molecular Biology

Topic: *Cis-/Trans*-splicing, Circular RNA, RNA Editing, Post-transcriptional Regulation, Expression Quantitative Trait Loci

Strategy: Omics Data Analysis Based on Computer Science and Statistics, Experimental Validation Based on Molecular Biology

Disease: Neuropsychiatric Disorders, Glioma, Breast Cancer

Major Honors and Awards

1998	Academic Paper Awards from the Image Processing and Pattern Recognition (IPPR) Society
1999-2000	Academia Sinica Post-doctoral Fellowship

2001	Post-doctoral Research Award of National Health Research Institutes (NHRI, Taiwan)
2007	Wu Ta-Yuo Memorial Award, National Science Council
2007	Academia Sinica Research Award for Junior Research Investigators
2012	Pius XI Medal, the Pontifical Academy of Sciences, Vatican
2014-2018	Project for Excellent Junior Research Investigators Award, Ministry of Science and Technology, Taiwan
2004, 2006, 2010, 2016, 2020, and 2023	Significant publications of Academia Sinica

Distinguishing between Intragenic *Trans*-spliced and Circular RNAs by Long-read Sequencing

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Transcriptionally non-co-linear (NCL) transcripts can arise from *trans*-splicing (*trans*-spliced RNA or "ts-RNA") or *cis*-backsplicing (circular RNA or "circRNA") events. *Trans*-splicing occurs between two or more separate pre-mRNAs derived from a single gene or different genes. *Cis*-backsplicing occurs within a single pre-mRNA with a covalently closed loop structure characterized by a back-splice junction (BSJ). While numerous circRNAs have been detected in various species, ts-RNAs remain largely uninvestigated. Detecting ts-RNAs is often interfered by experimental artifacts, circRNAs and genetic rearrangements. Particularly, intragenic ts-RNAs, which are derived from separate precursor mRNA molecules of the same gene, are often mistaken for circRNAs through analyses of RNA-seq data. Here we developed a bioinformatics pipeline (NCLscan-hybrid), which integrated short and long RNA-seq reads to minimize false positives and proposed out-of-circle and rolling-circle long reads to distinguish between intragenic ts-RNAs and circRNAs. Combining NCLscan-hybrid screening and multiple experimental validation steps successfully confirmed that four NCL events, which were previously regarded as circRNAs in databases, originated from *trans*-splicing. CRISPR-based endogenous genome modification experiments further showed that flanking intronic complementary sequences can significantly contribute to ts-RNA formation, providing an efficient/specific method to deplete ts-RNAs. We also experimentally validated that one ts-RNA (ts-ARFGEF1) played an important role for p53-mediated apoptosis through affecting the PERK/eIF2a/ATF4/CHOP signaling pathway in breast cancer cells. Our study thus describes both bioinformatics procedures and experimental validation steps for rigorous characterization of ts-RNAs, expanding future studies for identification, biogenesis, and function of these important but understudied transcripts. Furthermore, on the basis of nanopore long-read sequencing with circRNA enrichment, we developed an integrative resource (FL-circAS) and identified 884,636 BSJs with 1,853,692 full-length circRNA isoforms in human and 115,173 BSJs with 135,617 full-length circRNA isoforms in mouse. FL-circAS also provides multiple circRNA features related to circRNA expression at both the BSJ and isoform levels, biogenesis, and function. FL-circAS thus serves as the first resource for discovering full-length circRNAs at the isoform level, providing user-friendly interfaces for browsing, searching, analyzing, and downloading data. FL-circAS is freely available at https://cosbi.ee.ncku.edu.tw/FL-circAS/.

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Education

2003-2008	Ph.D., Medical Sciences, Tzu Chi University, Hualien, Taiwan
2009-2012	Postdoctoral, Cell and Cancer Biology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Research and Professional Positions Held in Chronological Sequence

2013-2016	Assistant Professor, Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan
2017-2018	Visiting Research Scholar, Department of Pathology, Duke University Medical Center, Durham, NC, USA
2016-2019	Associate Professor, Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan
2019-present	Professor, Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan
2022-2023	Visiting Scholar, Cancer Immunology and Virology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
2023-present	Distinguished Professor, Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan.

Research Interests

Molecular and Cellular Biology, Tumor Metastasis and Signal Transduction, Cancer Metabolism, Cancer immunotherapy

Major Honors and Awards

2014	Academic Research Award for Young Scholar, Taipei Medical University
2014	Academic Research Award for Excellent Researcher, Taipei Medical University
2015	Academic Research Award for Young Scholar, Taipei Medical University
2017	Cancer Research Excellence Award for Young Researcher, Taiwan Oncology Society
2018	Ta-You Wu Memorial Award, National Science and Technology Council, Taiwan

Exploring the Metabolic Gene Regulatory Network in Neuroendocrine Prostate Cancer for Potential Immunotherapy Applications

Yen-Nien Liu
劉晏年

College of Medical Science and Technology, Taipei Medical University, Taiwan

Neuroendocrine prostate cancer (NEPC) is a highly aggressive form of prostate cancer, marked by significant changes in metabolism and the ability to evade the immune system. Our research delves into the metabolic gene regulatory network of NEPC, examining the molecular mechanisms that connect neuroendocrine differentiation (NED) with metabolic changes caused by androgen deprivation therapy (ADT), and their effects on immunotherapy. We identified key metabolic regulators, which are affected by ADT and drive NED and resistance to therapy in prostate cancer. We also explored the interactions within the tumor microenvironment (TME) that influence NED, highlighting these regulators as potential therapeutic targets. Additionally, our research demonstrates how a regulator of calcium signaling and NED affects the hypoxia pathway, emphasizing the role of hypoxic conditions in the TME on NED and epithelial-mesenchymal transition (EMT). We are also working on developing synthetic gene circuits for cancer immunotherapy, specifically targeting NEPC. These circuits are designed to differentiate NEPC cells from normal cells and program them to produce therapeutic proteins, thereby initiating effective tumor-localized combination immunotherapy. Our study unravels the complex metabolic and molecular networks driving NED in prostate cancer, offering promising therapeutic targets and strategies for immunotherapy to manage aggressive NEPC.

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Education

1987	B.S. Biology, Tung-Hai University, Taiwan
1996	Ph.D., Molecular/Cellular Biology, National Yang-Ming University, Taiwan

Research and Professional Positions Held in Chronological Sequence

2023-2024	Visiting Scholar at Stanford University (Genetics), Stanford, CA
2022-present	Investigator - Institute of Molecular and Genomic Medicine, National Health Research Institutes, Taiwan (Immuno-oncology Research)
2011-2022	Associate Investigator - Institute of Molecular and Genomic Medicine, National Health Research Institutes, Taiwan (Cancer Biology and Translational Medical Research)
2016-2019	Health Product Development Consultant - Hi-Q Marine Biotech International Ltd., Taiwan
2012-present	Adjunct Professor, Tung-Hai University, Taiwan (Molecular and Genomic Medicine Lecture)
2011-2015	Adjunct Assistant Professor, National Taiwan University, Taiwan (Cancer Biology Lecture)
2004-2011	Assistant Investigator - Division of Molecular and Genomic Medicine, National Health Research Institutes, Taiwan (Molecular Oncology and Genomic Medicine Study)
2002-2004	Research Assistant Professor - Northwestern University Medical School, Chicago, IL (Hematology and Oncology Medical Research, Co-PI for Clinical/Translational Study)
2001-2002	Scientist/Biochemist - Lawrence Berkeley National Laboratory, Berkeley, CA (DNA Repair and Telomere Structure)
1999-2001	Postdoctoral Fellow in Biochemistry - Lawrence Berkeley National Laboratory, Berkeley, CA (Molecular Mechanism of Telomere Maintenance)
1996-1999	Postdoctoral Fellow in Biochemistry - Los Alamos National Laboratory, Los Alamos, NM (Molecular Regulation in DNA Damage Repair)
1987-1990	Instructor, Tung-Hai University, Taiwan (Biology, Genetics and Immunology)

Research Interests

Dr. Hsu's research interest is to investigating the oncogenic mechanisms that induce DNA damage, chromosomal instability, mitotic catastrophe, neoplastic multinucleation, tumor progression, metastasis and microenvironment. Dr. Hsu's current works focus on the fundamental mechanism of MCT-1 oncogene in promoting of tumor metastasis and malignant microenvironment. Her laboratory has identified the oncogenic kinase pathways involving the microRNA biogenesis, EMT signaling activation and tumor progression/metastasis. Dr. Hsu has confirmed the clinical relevance of the MCT-1 activation in different types of aggressive breast cancers and lung carcinoma. Her laboratory also establishes a unique *ex vivo* three dimensional dissection of tumorigenic microenvironment to analyze the therapeutic effect and utilizes an *in vivo* animal work to demonstrate the tumorigenic outcomes upon gain or loss of the oncogenic activity. These findings have good prospects to develop new diagnosis and effective therapeutic methods that administrate aggressive chemo/radio-resistant carcinomas.

Major Honors and Awards

2008-2011	NRPGM Grant Award, NSC
2007-2010	Young Investigator Career Development Award, NSC
2001	Telomere Travel Award Given by the Telomere and Telomerase Meeting, CSHL
1996	1ST Prize of Dr. Chien-Tien Hsu's Science Award Given by the Chinese Society of Cell and Molecular Biology, Taiwan
1989-1996	Pre-doctoral Fellowship, National Yang-Ming University, Taipei, Taiwan

The Novel Targets and Combinatorial Therapies for Treatment of the Aggressive Breast Cancer

Hsin-Ling Hsu
徐欣伶

Institute of Molecular and Genomic Medicine, National Health Research Institutes, Taiwan

Rationale: Multiple copies in T-cell malignancy 1 (MCT-1) is a prognostic biomarker for aggressive breast cancers. Overexpressed MCT-1 stimulates the IL-6/IL-6R/gp130/STAT3 axis, which promotes epithelial-to-mesenchymal transition and cancer stemness. Because cancer stemness largely contributes to the tumor metastasis and recurrence, we aimed to identify whether the blockade of MCT-1 and IL-6R can render these effects and to understand the underlying mechanisms that govern the process.

Methods: We assessed primary tumor invasion, postsurgical local recurrence and distant metastasis in orthotopic syngeneic mice given the indicated immunotherapy and MCT-1 silencing (shMCT-1).

Results: We found that shMCT-1 suppresses the transcriptomes of the inflammatory response and metastatic signaling in TNBC cells and inhibits tumor recurrence, metastasis and mortality in xenograft mice. IL-6R immunotherapy and shMCT-1 combined further decreased intratumoral M2 macrophages and T regulatory cells (Tregs) and avoided postsurgical TNBC expansion. shMCT-1 also enhances IL-6R-based immunotherapy effectively in preventing postsurgical TNBC metastasis, recurrence and mortality. Anti-IL-6R improved helper T, cytotoxic T and natural killer (NK) cells in the lymphatic system and decreased Tregs in the recurrent and metastatic tumors. Combined IL-6R and PD-L1 immunotherapies abridged TNBC cell stemness and M2 macrophage activity to a greater extent than monotherapy. Sequential immunotherapy of PD-L1 and IL-6R demonstrated the best survival outcome and lowest postoperative recurrence and metastasis compared with synchronized therapy, particularly in the shMCT-1 context. Multiple positive feedforward loops of the MCT-1/IL-6/IL-6R/CXCL7/PD-L1 axis were identified in TNBC cells, which boosted metastatic niches and immunosuppressive microenvironments. Clinically, *MCT-1^{high}/PD-L1^{high}/CXCL7^{high} and CXCL7^{high}/IL-6^{high}/IL-6R^{high} expression patterns predict worse prognosis and poorer survival of breast cancer patients.*

Conclusion: Systemic targeting the MCT-1/IL-6/IL-6R/CXCL7/PD-L1 interconnections enhances immune surveillance that inhibits the aggressiveness of TNBC.

Keywords: MCT-1, IL-6/IL-6R, CXCL7/CXCR2, PD-L1, Immunotherapy

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Education

1983-1990	M.D., June, Taipei Medical College, Taipei, Taiwan

Research and Professional Positions Held in Chronological Sequence

1990-2005	Resident/ Chief Resident/ Fellow/ Attending Neurologist, Department of Neurology, Taipei Municipal Jen-Ai Hospital, Taipei, Taiwan
2000-2001	Research fellow, Department of Neurology, Washington University, School of Medicine, St. Louis, Missouri, USA
2002-2003	Instructor, Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan
2004-present	Instructor/ Assistant Professor/ Associate Professor/ Professor, Department of Neurology, Taipei Medical University, Taipei, Taiwan
2005-2008	Attending Neurologist, Department of Neurology, Taipei Medical University Hospital, Taipei, Taiwan
2008-2018	Chief, Department of Neurology, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan
2008-present	Attending Neurologist/Consultant, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan
2015-2024	Director, Dementia Center, Taipei Medical University-Shuang Ho Hospital, New Taipei City, Taiwan
2016-2020	Vice superintendent in research affair, Taipei Medical University-Shuang Ho Hospital, New Taipei City, Taiwan
2017-2023	Director of PhD program in Medical Neuroscience, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan
2018-2024	Vice superintendent, Taipei Neuroscience Institute, Taipei Medical University, Taiwan
2018-2021	President, Taiwan Dementia Society
2020-2024	Vice dean, Medical College, Taipei Medical University, Taiwan
2021-2023	President, Taiwan Neurological Society
2021-present	Supervisor, Taiwan Dementia Society
2023-Present	Executive Supervisor, Taiwan Neurological Society
2024-present	Dean, Medical College, Taipei Medical University, Taiwan

Research Interests

Clinical Neurology, Molecular Neurology, Dementia, Clinical Trial

Mechanisms of Vascular Dementia--Risk Factors, Glymphatics, Neuro-inflammation, Senescence

Chaur-Jong Hu
胡朝榮

College of Medicine, Taipei Medical University, Taiwan

Vascular dementia or vascular cognitive impairment (VCI) following Alzheimer disease (AD) is the second common cause of dementia. Along with aging, the incidences of coexisting of AD pathology, in terms of amyloid and tau accumulation and pathological markers of cerebral vascular diseases are increasing. However, the mechanisms and risk factors of VCI are not totally clear and there is no effective treatment for VCI currently. Our team established a post-stroke cognitive impairment (PSCI), which is the most precise diagnosis of VCI cohort and explored the roles of glymphatics, neuro-inflammation and senescence in VCI.

A total of 588 ischemic stroke patients had been enrolled. Among them, patients with post-stroke cognitive impairment (PSCI) exhibited higher rates of old age, heart diseases, and diabetes and lower body mass index (BMI). The blood-based biomarkers study revealed significantly elevated plasma levels of sCD137 and CSCL13 in PSCI patients. The senescence-associated secretory phenotype (SASP) index scores were different between groups. PSCI patients had a significantly higher percentage of elevated cognitive impairment-related polygenic risk scores (PRS).

In the chronic cerebral hypoperfusion, mouse bilateral common carotid artery occlusion (BCCAO) model, after injection of Gd-contrast median into 4th ventricle, the contrast medium wash-in rate and wash-out rate in brain were reduced and contrast medium remains at 300 min after injection increased. It implicates glymphatic impairment in BCCAO animals. In human study, we found glymphatic function, measured by MRI diffusion-tensor-image analysis along the perivascular space (DTI-ALPS) index is correlated with cognitive functions among PSCI patients and it might be associated with hemodynamic parameters, including resistance index and pulsatility index in middle cerebral arteries. These results support hemodynamic changes induced by cerebrovascular diseases play a role in glymphatic dysfunctions and glymphatic dysfunction is attributed to cognitive impairment.

In acute ischemic stroke and chronic cerebral hypoperfusion (CCH) models, we identified key interactions between hyper-reactive microglia with glymphatic structures. Our findings highlighted the impairment of glymphatic clearance, decreased astrocytic endfeet coverage on blood vessels, and reactive microglial infiltration into the perivascular space post-stroke. In stroke models with rehabilitation therapy and microglia-specific transcriptomic analyses, we identified TRIM5 as a crucial mediator which exhibited the most prominent regulation, localizing to microglial cells interacting with glymphatic structures. Using TRIM5 knockout mice, we demonstrated that the absence of TRIM5 ameliorated stroke-induced motor, cognitive, and emotional impairments.

In *in vitro* ischemia model, oxygen-glucose deprivation/reoxygenation (OGD/R) significantly increased senescence-associated beta-galactosidase (SA-ß-gal) staining in astrocytes and microglia, with minor effects on neurons. *In vivo* CCH model, bilateral carotid artery stenosis (BCAS) induced elevated p21 expression accompanied by increased double-positive p21/Iba1 and p21/NeuN cells. BV2 cell-conditioned media/exosomes induced senescence and upregulated SASP in naïve BV2 cells. The senolytic cocktail dasatinib and quercetin (D+Q) selectively eliminated senescent BV2 cells. These findings highlight the induction of cellular senescence in stroke and VCI models.

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Education

1976-1980	B.S., School of Pharmacy, National Taiwan University, Taiwan
1980-1983	M.S., Graduate Institute of Pharmacology, National Taiwan University, Taiwan
1989-1993	Ph.D., Graduate Institute of Pharmacology, National Taiwan University, Taiwan

Research and Professional Positions Held in Chronological Sequence

1980-2001	Teaching Assistant/Lecturer/Associate Professor, Dept. Pharmacology, College of Medicine, National Taiwan University.
1995-1996	Visiting Scientist, University of Texas Medical Branch at Galveston, Texas, USA.
2002-	Professor, Dept. Pharmacology, College of Medicine, National Taiwan University.
2011-	Professor, Graduate Institute of Brain and Mind Sciences, National Taiwan University.
2014-2020	Director, Graduate Institute of Brain and Mind Sciences, National Taiwan University.
2015-	Professor, Graduate Institute of Acupuncture Science, China Medical University.
2022-	Professor, School of Medicine, National Tsing Hua University.

Research Interests

In earlier years at NTU, we employed electrophysiological approaches in midbrain slices containing the periaqueductal gray (PAG) [1], a crucial area for initiating the descending analgesic pathway, to elucidate the cellular mechanisms of analgesic compounds. They include opioids [2], new ligands of nociception/orphanin FQ receptors [3-7], the 4th opioid receptor member, and orexins [8-12]. We also elucidated the neuroplasticity changes in the PAG of animals with neuropathic pain [13, 14]. With the enrollment of an anesthesiologist, we have also revealed the spinal analgesic mechanism of gabapentin in neuropathic pain [15, 16].

The studies derived from the finding that orexins can induce analgesia via orexin 1 receptor-mediated endocannabinoid (eCB) synthesis in the PAG [9] are noteworthy. We found that this orexin-initiated eCB signaling in the PAG can contribute to stress-induced analgesia [10, 11], and surprisingly, also to electroacupuncture-induced analgesia via median nerve stimulation [12, 17]. Interestingly, this signaling also existed in the ventral tegmental area, contributing to stress-induced extinguished cocaine-seeking [18, 19].

Since 2019, we have launched a drug development project inspired by a clinical case study where a local herb effectively alleviated intractable motor tics [20]. From this herb, an active constituent, hispidulin, was identified [21, 22], which primarily acted as a positive allosteric modulator (PAM) of the α6 subunit-containing $GABA_A$ receptors (α6$GABA_A$Rs) in the cerebellum of animal models mimicking schizophrenia [23, 24]. This finding later led to the formation of an international collaborative consortium aiming to develop α6$GABA_A$R-selective PAMs as the first-in-class novel therapy for several neuropsychiatric disorders, including schizophrenia [23, 25], migraine [26-28], and essential tremor [29]. In addition to having European and US patents approved, we have completed a comprehensive review on α6$GABA_A$Rs, published in Pharmacological Reviews in 2022, where several potential indications of α6$GABA_A$R-selective PAMs were proposed [30].

Recently, we achieved a breakthrough study appreciated by the Annals of Neurology [31]. We found that knocking down of the *Slitrk1* gene, a risk gene of Tourette syndrome (TS), in the striatum of adult mice recapitulated the motor tic-like behaviors of TS patients. These mice displayed impaired cholinergic neuronal activity and a dysregulated dopamine system in the striatum. These results suggest that the Slitrk 1 protein in striatal cholinergic neurons can bridge cholinergic and dopaminergic systems, two important pathogenic mechanisms, in TS. α6$GABA_A$R-selective PAMs displayed a preliminary positive result when tested in *Slitrk1*-KD mice, suggesting their therapeutic potential in TS.

Major Honors and Awards

2001	EPHAR Poster Award, 3rd EPHAR Congress, Federation of European Pharmacological Societies, Lyon, France
2002	IUPHAR Highly Commended Young Investigator Award Finalist, The XIV World Congress of Pharmacology, IUPHAR, San Francisco, USA
2004	Research Achievement Award, National Taiwan University, Taipei, Taiwan.
2004	Distinguished Research Award, Taiwan Pharmacology Society, Taiwan
2005- 2010	Innovative Research Grant Award, National Health Research Institute, Taiwan.
2013	NHRI Extramural Grant award (for grantee awarded three times), National Health Research Institutes, Taiwan
2017	Future Technology Breakthrough Award, Minister of Science and Technology, Taiwan
2018	Outstanding Research Award, Minister of Science and Technology, Taiwan
2019	The 15th Tien Te Lee Outstanding Biomedical Award, Taiwan
2020	Tu Tsung-Ming Lecture Award, 113th Annual Meeting of Formosan Medical Association, Taipei, Taiwan

Unlocking Promise: α6GABA$_A$R Positive Modulators for Neuropsychiatric Disorders

Lih-Chu Chiou
邱麗珠

Department of Pharmacology, College of Medicine, National Taiwan University, Taiwan

Over the past decade, our team embarked on a drug development project inspired by the finding that a local herb (*Clerodendrum inerme*) significantly reduced a patient's intractable motor tics (Fan et al., 2009). We later identified a flavonoid, hispidulin, as an active constituent using animal models mimicking hyperlocomotion and disrupted prepulse inhibition (PPI), the endophenotype in patients with tic disorders and schizophrenia. Using pharmacological and anatomical approaches, we also demonstrated that hispidulin exerted its PPI-restoring effect mainly via acting as a positive allosteric modulator (PAM) of the α6 subunit-containing GABA$_A$R (α6GABA$_A$R) in the cerebellum (Chiou et al., 2018).

GABA$_A$Rs are pentameric (2α, 2β and 1γ/ δ) subunit-forming ligand-gated Cl^- channels mediating inhibitory neurotransmission. Among various GABA$_A$R subtypes consisting of different combinations of 19 (α1-6, β1-3, γ1-3, δ, ε, π, θ and ρ1-3) identified subunits, were understudied due to their limited distribution in the cerebellum and lack of selective ligands. However, several pyrazoloquinolinone compounds (PQs) were identified in 2013 as α6GABA$_A$R-selective PAMs. They bind to an interface (α+β–) of GABA$_A$Rs different from that for GABA (α-β+) or benzodiazepine BDZ (α+γ-).(Knutson et al., 2018) Therefore, PQ compounds are potential drug candidates without BDZ-like side effects. Our findings on hispidulin led to an international drug development consortium aiming to develop PQ compounds as a novel therapy for neuropsychiatric disorders. Using PQ Compound 6 and its deuterated derivative, DK-I-56-1, we have provided proof-of-concept in animal models mimicking schizophrenia (Chiou et al., 2018; Lee et al., 2022), migraine (Fan et al., 2018; Tzeng et al., 2021), and essential tremor (Huang et al., 2023). In a comprehensive review article targeting α6GABA$_A$Rs (Sieghart et al., 2022), we have proposed several other potential indications of α6GABA$_A$R-selective PAMs in neuropsychiatry, including Tourette syndrome (TS). In the recent NHRI-funded study, we have established an animal model that can capitulate motor tics manifested in TS patients by bilateral microinjection of *Slitrk1* siRNA in the dorsal striatum of adult mice. The motor tics in these striatal *Slitrk1*-knockdown mice may be due to their lower striatal acetylcholine levels owing to fewer Slitrk1-containing cholinergic interneurons, leading to lower evoked, but higher tonic, dopamine levels, downregulated dopamine transporter, and D2 receptor hyper-responsiveness in the striatum (Du et al., 2024). A positive preliminary result prompts us to explore the therapeutic potential of PQ compounds in TS further using this model.

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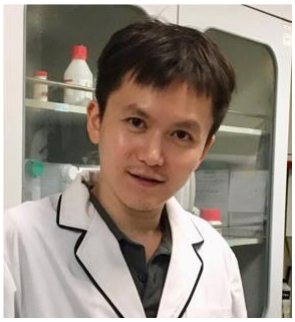

Education

2000-2005	B.Sc. (Hon), Pharmacology, University of British Columbia, Canada
2003	Research Internship, Merck Frosst Centre for Therapeutic Research, Canada
2004	Research Internship, Cardiome Pharma Corp. Canada
2005-2011	Ph.D., Neuroscience, University of British Columbia, Canada

Research and Professional Positions Held in Chronological Sequence

2011-present	Research Fellow, Translational Medicine Research Center, China Medical University Hospital, Taiwan
2012-2016	Assistant Professor, Graduate Institute of Clinical Science, College of Medicine, China Medical University, Taiwan
2016-present	Associate Professor, Graduate Institute of Biomedical Sciences, College of Medicine, China Medical University, Taiwan

Research Interests

Pain, Microglia, Blood-Brain Barrier, Stroke and excitotoxicity

Major Honors and Awards

2000	British Columbia (BC) Provincial Scholarship
2002	Charles and Jane Banks Scholarship in Science
2001-2004	UBC Undergraduate Scholarship Program
2001-2005	UBC Dean's Honour List Student
2004	NSERC Undergraduate Student Research Award
2002/2005	UBC Science Scholar Awarded to top 20 science students amongst all of UBC departments combined (incl. math, chemistry, physics, biology, statistics, and computer science)
2005	Esther R. Anderson Memorial Prize Ranked # 1 of Graduating Class (equivalence of *summa cum laude*)
2005	UBC Faculty of Medicine Undergraduate Research Award
2005	Canadian Stroke Network (CSN) Studentship
2005-2007	CIHR/MSFHR Strategic Training Scholarship in Neurobiology and Behaviour
2006-2007	NSERC Alexander Graham Bell Canada Graduate Scholarship Ranked # 1 of UBC Neuroscience (Master's & Junior Doctoral)

2007	Canadian Stroke Network (CSN) Poster Competition Winner
2006-2008	Michael Smith Foundation for Health Research (MSFHR) Junior Graduate Scholarship
2008	UBC Poster Competition Travel Award
2008	CIHR Neuroscience Poster Competition Travel Award
2009	Student Biotechnology Network (SBN) Genomics Forum Poster Competition Winner
2007-2010	CIHR Frederick Banting and Charles Best Canada Graduate Scholarship Ranked # 1 of UBC Neuroscience (Senior Doctoral)

Pain Caused by Citric Acid: from Drug Formulations to Animal Venoms and Beyond

Ted Weita Lai
賴威達

Graduate Institute of Biomedical Sciences, China Medical University, Taiwan

Citric acid is a common ingredient used in pharmaceutical formulations. Despite the widespread clinical use of these formulations, it remains unclear how citric acid causes pain when injected into patients. We identified ASIC1 as the key receptor used to detect injection-site pain caused by acid, and we showed that neutral citrate does not stimulate ASIC1; instead, citrate substantially potentiates ASIC1 by removing the inhibitory action of calcium on the extracellular side of the receptor. Given that injection-site pain is the primary complaint of patients receiving citrate-containing medical products, our data provide mechanistic insight into a common medical complaint and suggest a means of avoiding injection pain.

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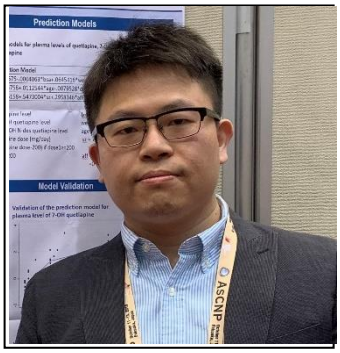

Education

1997-2004	M.D., Medicine, National Yang-Ming University, Taiwan
2012-2013	M.H.S., Mental Health, Johns Hopkins Bloomberg School of Public Health, USA
2013-2018	Sc.D., Epidemiology, Harvard Chan School of Public Health, USA

Research and Professional Positions Held in Chronological Sequence

2008-2010	Attending Psychiatrist, Department of Psychiatry, En Chu Kong Hospital, Taiwan
2017-2019	Attending Psychiatrist, Department of Psychiatry, Taipei City Psychiatric Center, Taiwan
2018-2019	Adjunct Assistant Professor, Department of Psychology, National Cheng-Chi University, Taiwan
2019-Present	Adjunct Assistant Professor, School of Medicine, National Yang Ming Chiao Tung University, Taiwan
2021-Present	Adjunct Assistant Professor, Institute of Behavioral Medicine, National Cheng Kung University, Taiwan
2019-Present	Assistant Investigator Attending Physician, Center for Neuropsychiatric Research, National Health Research Institutes, Taiwan

Research Interests

Dr. Lin is a board-certified psychiatrist in Taiwan and also a genetic epidemiologist. He received his ScD degree from the Department of Epidemiology, Harvard Chan School of Public Health. Dr. Lin's research interests lie in precision psychiatry and genetic epidemiology of psychiatric, substance use, and neurocognitive disorders. His work centers on analyzing large-scale GWAS and epidemiological data to identify the genetic determinants of psychiatric disorders, to examine the genetic overlap between different phenotypes, and to establish causal relationships between genetic and environmental factors, as well as clinical phenotypes. He also aims to utilize neurogenomics, informatics, and artificial intelligence to promote precision psychiatry and achieve better treatment outcomes.

Major Honors and Awards

2013	Delta Omega Honor Society Alpha Chapter, Johns Hopkins School of Public Health
2013-2016	The MOE Technologies Incubation Scholarship, Ministry of Education, Taiwan

2015-2016	Rose Traveling Fellowship in Chronic Disease Epidemiology and Biostatistics, Harvard University
2022	Dr. Paul Janssen Research Award, TSBPN
2020-2024	Teaching Excellence Awards, National Yang Ming Chiao Tung University

Polygenic Prediction of Antidepressant Treatment Response

Yen-Feng Lin
林彥鋒

Center for Neuropsychiatric Research, National Health Research Institutes, Taiwan

Antidepressant response is a complex trait influenced by both genetic and environmental factors. However, despite decades of research, the specific genetic variations that contribute to antidepressant response and treatment-resistant depression (TRD) remain largely unknown. Polygenic risk scores (PRS), allowing for the capture of cumulative risk across the genome, are considered as a more useful tool than single SNPs or genes for multifactorial complex traits, such as antidepressant response and TRD. In addition, PRSs can be used to predict relevant traits and assess the genetic correlation of different phenotypes. However, the use of PRS for predicting antidepressant response has yielded inconclusive results. Further research with larger sample sizes and standardized outcome measures is needed to determine the utility of PRS in predicting antidepressant response and TRD.

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Education

1985-1992	M.D., Kaohsiung Medical College, Kaohsiung, Taiwan
1999-2003	Ph.D. in Bioengineering, University of Washington, Seattle, WA, USA

Research and Professional Positions Held in Chronological Sequence

1992-1994	Military Doctor, Tong-In Military Hospital, Tong-In, Taiwan
1994-1996	Resident in Surgery, Chang-Gang Memorial Hospital, Kaohsiung, Taiwan
1996-1999	Senior/Chief Resident, Cardiovascular surgery, National Taiwan University Hospital, Taipei, Taiwan
2004-2006	Research Fellow, Harvard Medical School/MIT, Boston, MA, USA
2006-2014	Affiliate, Associate & Full Professor, Graduate Institute of Clinical Medicine, National Cheng Kung University, Tainan, Taiwan
2006-2014	Attending Surgeon, Cardiovascular Surgery Division, NCKU Hospital
2009-2014	Joint Assistant, Associate Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
2011-present	Affiliate Associate, Full Professor, Bioengineering Department, University of Washington, Seattle, USA
2014-present	Professor, Graduate Institute of Medical Genomics and Proteomics, NTU College of Medicine
2014-2019	Affiliate Attending Surgeon, Cardiovascular Surgery Division, NTU Hospital
2014-2019	Associate, full Research Fellow, IBMS, Academia Sinica
2017-present	Visiting Professor, Cardiology and Stem Cell & Regenerative Medicine Center, University of Wisconsin, Madison
2018-present	Chief, Division of Cardiovascular and Metabolic Diseases, IBMS
2019-present	Distinguished Research Fellow, IBMS
2022-present	Chair, Institutional Review Board (IRB) Biomedical Committee, Academia Sinica, Taipei, Taiwan

Research Interests

Stem cells, regenerative medicine, nanomedicine, translational biology, cardiovascular

Major Honors and Awards

2004-2006	Postdoctoral fellowship, American Heart Association (AHA)
2005	Poster award, Current Progress in Tissue Engineering and Regenerative

	Medicine, C.I.M.I.T. at MIT and Harvard Medical School, Boston, MA
	Finalist, Melvin L. Marcus Young Investigator Award in Cardiovascular Science, AHA
	Finalist, Northwestern U. Feinberg School of Medicine Cardiovascular Young Investigators' Forum
2010	Outstanding undergraduate researcher advisor award, National Science Council, Taiwan
2011	National Innovation Award, Institute of Biotechnology and Medicine Industry, Taiwan
2012	Outstanding Research Award, National Science Council, Taiwan
2013	National Innovation Award, Institute of Biotechnology and Medicine Industry
	Top 1 translational researcher in 2012 (junior faculty category), Nature Biotechnology
	International Fellow of American Heart Association (FAHA), Council on Cardiovascular Sciences, American Heart Association
	Best paper in 2012, National Cheng Kung University College of Medicine, Tainan, Taiwan
2014	Chair in Biotechnology, Taiwan Bio-Development Foundation (TBF)
2015	Outstanding Research Award, Ministry of Science and Technology, Taiwan
	Selected Significant Research Achievements of Academia Sinica
	Principle Investigator, Taiwan Human Diseased iPSC Service Consortium, MoST, Taiwan
2016	Extramural grant award, National Heath Research Institutes (NHRI), Taiwan
	Selected Significant Research Achievements of Academia Sinica
2017	Principle Investigator, Taiwan Heart Tissue Chip Project, MoST, Taiwan
	World Leading Young Scientist, Society of Polymer Science, Japan
	TECO Award, TECO Technology Foundation, Taiwan
2018	Selected Significant Research Achievements of Academia Sinica
	Project for MoST Research Fellow, Ministry of Science and Technology
2020	Healthy Longevity Global Grand Challenge Competition, National Academy of Medicine, USA
2021	Distinguished Alumnus Award, Kaohsiung Medical University, Taiwan
	Selected Significant Research Achievements of Academia Sinica
	Project for MoST Research Fellow, Ministry of Science and Technology
2022	Selected Significant Research Achievements of Academia Sinica
	Merit Award, National Heath Research Institutes (NHRI), Taiwan
2023	Selected Significant Research Achievements of Academia Sinica
	Co-President, Academy of Cardiovascular Research Excellence (ACRE)-Taiwan Chapter
	Cofounder and Council Member, International Society for Heart Research-Southeastern Asia (ISHR-SEA)
	Founder and President, Taiwan Circulation Research Society (TCRS)

Microbiota-Derived Metabolites and Cardiac Resilience: Unveiling the Gut-Heart Connection

Patrick Ching-Ho Hsieh
謝清河

Institute of Biomedical Science, Academia Sinica, Taiwan

The intricate relationship between gut microbiota and cardiac health has emerged as a captivating frontier in cardiovascular research. In this presentation, I delve into the findings of three studies from our group, each shedding light on distinct aspects of this remarkable gut-heart connection.

In our first study, we investigate how gut microbiota depletion affects immune cell composition and the heart's repair capacity following myocardial infarction (MI). This study underscores the pivotal role of gut microbiota-derived short-chain fatty acids (SCFAs) in maintaining host immune composition and enhancing post-MI outcomes (Tang et al., *Circulation*, 2019).

The second study delves into the influence of gut microbiota on cardiac remodeling in response to pressure overload stress. Here, we demonstrate the essential role of acetate and propionate as mediators in gut microbiota-modulated cardiac mechanics, offering a potential therapeutic approach to enhance cardiac health in the presence of dysbiosis (Lin et al., *Theranostics*, 2022).

In our final study, we explore the role of butyrate-producing bacteria in post-MI responses. This research reveals how these microorganisms produce beta-hydroxybutyrate, providing support for cardiac function post-MI. Insights from this study offer new perspectives on the gut microbiota's role in the cardiac recovery process (Chen et al., *Nat Communications*, 2023).

Through these studies, we unveil the potential of utilizing the gut microbiota and their metabolites as therapeutic targets to enhance cardiac repair, adaptation, and protection.

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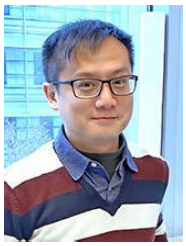

Education

1997-2001	B.S., Department of Animal Science, National Taiwan University, Taiwan
2001-2003	M.S., College of Medicine, National Cheng Kung University, Taiwan
2005-2011	Ph.D., College of Medicine, National Taiwan University, Taiwan

Research and Professional Positions Held in Chronological Sequence

2011	Postdoctoral Fellow, National Center for Genome Medicine, Academia Sinica, Taiwan
2012-2013	Academia Sinica Regular Postdoctoral Fellow, Taiwan
2014-2015	Academia Sinica Distinguished Postdoctoral Scholar, Taiwan
2017	Postdoctoral Researcher, Department of Medicine, the University of Chicago, USA
2018-2022	Assistant Professor, Department of Biological Science and Technology / Joint Assistant Professor, Institute of Bioinformatics and Systems Biology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan.
2019-now	Joint Appointment Assistant Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan
2022-now	Associate Professor, Department of Biological Science and Technology, / Joint Associate Professor, Institute of Bioinformatics and Systems Biology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan.

Research Interests

My research is dedicated to identifying characteristics of individual immune status and developing personalized treatment strategies based on integrative genomic data analysis. We employ advanced single-cell genomic tools to uncover details of peripheral immune perturbations, which provide insights into immune responses and serve as critical variables influencing the efficacy of immunotherapies. Utilizing integrative biomedical data and sophisticated immune-pharmaco-genomics platforms, we explore peripheral immune perturbations associated with human diseases (*Circulation Research*, 2015; *BMJ*, 2015; *JAMA Network Open*, 2021; *Clinical Translational Allergy*, 2022; *Clinical and Translational Medicine*, 2022; *Circulation*, 2023). My methodology encompasses extensive single-cell meta-analyses and the integration of multimodal data to pinpoint genomic determinants crucial for guiding therapeutic strategies.

Major Honors and Awards

2012	Thesis Award of Tsung-Ming Tu, Taiwan

2012	The poster price winners of the Drug Hypersensitivity Meeting 5, Germany
2012	FOCIS Travel Award of Federation of Clinical Immunology Societies, Canada
2012	Excellent Thesis Award from National Taiwan University, Taiwan
2014	Distinguished Postdoctoral Scholarship, Academia Sinica, Taiwan
2018	Tsung-Ming Tu's YOUNG INVESTIGATOR AWARD, The Pharmacological Society in Taiwan, Taiwan (杜聰明博士年輕學者獎)
2018	YOUNG INVESTIGATOR AWARD, The 12th International Kawasaki Disease Symposium, Japan (國際川崎病研討會年輕研究學者獎)
2018-2022	Bio-ICT (Information and Communications Technology), Junior Chair Professor, National Chiao Tung University, Taiwan (國立交通大學 Bio-ICT 青年講座教授)
2019	JCA-CHAAO Award (Category: Cancer research using artificial intelligence technologies on the frontiers of medical science), Japanese Cancer Association, Japan
2021	Selected member of the Clinical Translational Research Network (CTRN), 10x Genomics, USA
2023	The Young Scholars' Creativity Award, Foundation for the Advancement of Outstanding Scholarship, Taiwan (傑出人才發展基金會 第十一屆年輕學者創新獎)
2024	Professor Chen-Yuan Lee Outstanding Research Award, the Pharmacological Society in Taiwan (台灣藥理學會 李鎮源教授傑出研究獎)

Decoding Precise Immune Regulation and Human Diseases with Multimodal Single-Cell Analysis of Peripheral Immune Perturbations

Tai-Ming Ko
柯泰名

Department of Biological Science and Technology, College of Engineering Bioscience,
National Yang Ming Chiao Tung University, Taiwan

The complexity of immune regulation across various diseases necessitates advanced methodologies to parse immune response variability and optimize treatment efficacy. Dissecting individual immune statuses to develop personalized treatment strategies remains a substantial challenge. We utilize advanced single-cell genomic tools to deeply analyze peripheral immune perturbations, employing extensive meta-analyses and multimodal data integration to identify genomic determinants crucial for therapeutic guidance. Our investigations into Kawasaki disease (KD) and multisystem inflammatory syndrome in children (MIS-C) have uncovered aberrant neutrophil activation potentially regulated by the SPI1 gene, associated with severe cardiovascular outcomes. This insight directs our therapeutic targeting and drug repurposing strategies. Aiming to enhance precision medicine, we are developing personalized computational models—immune system digital twins—to simulate and predict immune responses. This initiative seeks to tackle the inherent complexity of immune dynamics, significantly advancing the personalization of treatment plans. Our upcoming research will explore the maternal immune impact on neonatal health, utilizing national health data to assess vaccination impacts and immune development. This aligns with our broader goal to refine digital twin technologies for more accurate prediction and management of immune-related diseases. By integrating state-of-the-art single-cell analysis in analyzing peripheral immune perturbations, our research is paving the way for transformative advances in personalized medicine.

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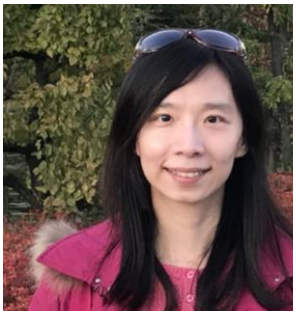

Education

1994-1998	B.S., Agronomy, National Taiwan University, Taiwan
1998-2000	M.S., Agronomy, National Taiwan University, Taiwan
2003-2009	PhD., Microbiology and Immunology, National Yang Ming University, Taiwan

Research and Professional Positions Held in Chronological Sequence

2000-2023	Scientist, Anawratha biotechnology Inc.
2009-2011	Post-doctoral fellow, Genomic Research Center, Academia Sinica, Taiwan
2012-2014	Post-doctoral fellow, Lineberger Comprehensive Cancer center, University of North Carolina at Chapel Hill, USA
2015-2020	Assistant Professor, Institute of Clinical Medicine, National Yang-Ming University Taiwan
2020~	Associate Professor, Institute of Clinical Medicine, National Yang-Ming Chiao Tung University Taiwan

Research Interests

Dr. Chen has been actively engaged in the fields of innate immunity, particularly in neutrophil and dendritic cell biology. Her current research focus revolves around investigating the role of pattern recognition receptors in host immunity, inflammatory diseases, and infectious diseases. Additionally, Dr. Chen is exploring the intricate interplay between dendritic cells and gamma-delta T cells, seeking to elucidate their collaborative mechanisms in immune responses.

Major Honors and Awards

2009	Outstanding Thesis award, “Chien-Tien Hsu Foundation” in the Chinese society of cell and molecular biology
2009	Distinguished Thesis award, “Tiente Lee Biomedical Foundation”
2009	Award of Long –Term Smile Contest, “Acer Foundation”
2012	Award of Postdoctoral Research Abroad Program multiple years, “National Science Council”, TW
2017	Project for Excellent Junior Research Investigators, Ministry of Science and Technology
2019	Outstanding Research Scholar Award, Chinese Society of Immunology
2020	College Student Research Creativity Award, Ministry of Science and Technology
2023	Wu Ho-Su TBF Taiwan Bio-development Foundation Medical Award

NLRP12, an Innate Immune Checkpoint:
Its Regulatory Properties in Pathogen Infections and Lupus Disease

Szu-Ting Jessica Chen
陳斯婷

Institute of Clinical Medicine, National Yang Ming Chiao Tung University, Taiwan

Innate immunity serves as the first line of host defense against infections. It also maintains physiological balance, influences the composition of the microbiota, and plays crucial roles in contexts of disease progression. These studies have significantly broadened our understanding of the innate immune system. Innate immunity is achieved by coupling different receptors or sensors to specific effector responses through activation of the selected signaling pathways. Those receptors known as pattern recognition receptors, initiate signaling cascades upon detecting various stimuli, including damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Among those receptors, members of the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) superfamily have recently gained tremendous attention. NLR proteins have rapidly emerged as central regulators of inflammation and immunity, with demonstrated relevance to human diseases. While certain NLR proteins are involved in canonical inflammasome activation, others regulate inflammatory signaling cascades beyond inflammasome assembly. NLRP12, a member of the NLR superfamily, is primarily expressed by cells of the myeloid lineage. NLRP12 limits DSS-induced colon inflammation and tumorigenesis through the negative regulation of canonical and noncanonical NF-κB signaling in an experimental colitis model. NLRP12 plays a crucial role in modulating inflammasome activation by physically interacting with NLRP3. This interaction serves to suppress NLRP3-dependent inflammasome activation. Therefore, the nonsense mutations in NLRP12 result in increased NLRP3 inflammasome activity due to the loss of confinement between NLRP12 and NLRP3. This leads to the spontaneous release of IL-1β, which has been implicated in various autoinflammatory diseases. Additionally, NLRP12 suppresses virus and nucleic acid-induced type I IFN production. This suppression occurs through the downregulation of *NLRP12* expression, which releases the confinement within the type I IFN receptor signaling during virus infection. Consequently, the host regulates innate immune signaling by modulating the expression levels of NLRP12, leading to an anti-viral response through increased IFN-I production. However, prolonged low NLRP12 expression results in excessive IFN-I production, facilitating the progression of inflammatory diseases, such as systemic lupus erythematosus. As a result, we propose that NLRP12 functions as an innate immune checkpoint, with its expression levels indicating immune resilience and homeostasis.

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Education

1997-2001	B.S., Zoology, National Taiwan University, Taiwan
2001-2003	M.S., Biochemistry, National Yang-Ming University, Taiwan
2003-2007	Ph.D., Molecular and Medical Pharmacology, University of California, Los Angeles, USA

Research and Professional Positions Held in Chronological Sequence

2007-2009	Postdoctoral fellow, University of California, Los Angeles, USA
2009-2012	Postdoctoral fellow, Whitehead Institute for Biomedical Research/MIT, USA
2012-2019	Assistant Investigator, National Health Research Institutes, Taiwan
2019-present	Associate Investigator, National Health Research Institutes, Taiwan

Research Interests

The goals of my lab are to study the regulation of cancer and tissue stem cells and to apply the finding in developing stem cell (SC)-based regenerative medicine and CSC-targeting therapy. We identify a mechanism by which PGE_2/prostaglandin E receptor 4 (EP_4) signaling regulate stem cells via extracellular vesicles (EVs)/exosomes. To apply the finding on cancer therapy, we find that EP_4 antagonism converts CSCs to chemotherapy-sensitive non-CSCs by triggering EV/exosome release and enhances tumor responses to chemotherapy (Int J Cancer. 2018;143:1440-1455; Cover Story). This finding holds implications for the treatment of chemoresistant carcinomas in the oncology clinic. Since the selective EV cargo sorting controls cellular component release from CSC, we further investigate the mechanism of EV cargo sorting of aggressive/metastatic tumor (EMBO Reports 2024;25: 2441-2478)

Blocking PGE_2/EP_4 signaling promotes SCs to release EVs/exosomes which carry SC properties. Non-SCs can acquire SC properties following ingestion of the indced SC EVs/exosomes (Stem Cells, 2017;35:425-444; J. Vis. Exp. 2017;124:e55736). The SC EV-mediated stemness transfer provides a new direction for regeneration medicine. We demonstrate that systemic administration of EP_4 antagonist-elicited MSC EVs/exosomes (iExo) induces hippocampal neuron regeneration and repairs deficiencies of cognition, learning and memory when being administered to mice following hippocampal damage (Stem Cells Transl Med. 2019;8:707-723; Stem Cells Transl Med. 2020;9:499-517). We develop iExo as regenerative medicine for brain damage/diseases. This invention has been patented and commented by Dr. Anthony Atala, said, "We look forward to seeing the successful

development of this technology in the treatment of brain injury and neurodegenerative diseases. We further transform the lab-based method for clinical application by developing clinical-grade SOP for iExo production. In 2022 June, the patents and the SOP have been transferred to Lumosa Therapeutics Co., Ltd with an exclusive license agreement, which allows to develop iExo-based therapy for human diseases. Meanwhile, our team has worked with Lumosa Therapeutics in an industry-academia collaboration to establish exosome production, analysis, and regulatory capabilities for clinical trials.

Major Honors and Awards

2002	The scholarship of the Medical Scholarship Foundation in Memory of Professor Albert Ly-young Shen, Taiwan
2002	Young Investigator Awards of International Congress on Hormonal Steroids and Hormones and Cancer, Japan
2009-2012	Postdoctoral Fellowship from Susan G. Komen for the Cure, USA
2019-2022	科技部優秀年輕學者研究計畫
2019	Ta-You Wu Memorial Award 吳大猷獎
2020-2023	吳大猷先生紀念獎研究計畫
2021	國家衛生研究院年輕學者研究獎
2022	外泌體專利技轉專屬授權

Exosomes in Stem Cell Homeostasis, Cancer, and Regenerative Medicine

Hua-Jung Li
李華容

Institute of Cellular and System Medicine, National Health Research Institutes, Taiwan

Exosomes are responsible for intercellular communication by passing molecules between cells. We found that promoting the release of induced exosomes (iExos) from stem cells leads to the transition of stem cells to a non-stem cell state. Upon release of iExos, basal mammary epithelial stem cells lose their properties. In contrast, after stem cell-derived iExos are taken up, mammary luminal cells are transformed into basal cell-like mammary epithelial stem cells and form mammary glands *in vivo*. The iExo-mediated stemness transfer provides a new direction for regenerative medicine. For example, an adult brain has limited regenerative capacity. As a result, brain injuries and neurodegenerative diseases often lead to impaired function in patients. Mesenchymal stem cells (MSCs) are a type of adult stem cells that can be isolated from various adult tissues and have been used in clinical trials for the treatment of human diseases. We found that EP4 antagonist-induced MSC exosomes (GW-iExos) have greatly increased therapeutic potential in rescuing a wide range of CNS lesions (e.g., neuronal degeneration and death, reactive astrocyte hyperplasia, widespread inflammation, and BBB disruption), whereas naïve exosomes derived from untreated MSCs are relatively inefficient in treating lesions that are commonly associated with brain injury, stroke, and a variety of neurological degenerative diseases such as AD and PD.

Similar to basal breast epithelial stem-like cells, breast cancer cells expressing a mesenchymal phenotype have cancer stem cell (CSC) properties and are often resistant to conventional chemotherapy. We can induce mesenchymal breast cancer stem cells to transform from a mesenchymal/CSC state to a more epithelialized non-CSC state by promoting the release of iExo, which removes CSC markers, mesenchymal markers, integrins, and drug efflux transporters. The induced exosome release increased the chemosensitivity of breast cancer cells, suggesting that, in combination with conventional chemotherapy, the exosome inducers could serve as an effective adjuvant for targeting cancer stem cells. On the other hand, we found that during tumor progression, tumor cells acquire an aggressive phenotype by altering protein interactions, which affect the sorting of exosomal cargoes, including proteins and nucleic acids. These findings have clinical implications for regenerative medicine and cancer therapy.

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Education

1983	B.S., Department of Public Health, College of Medical, National Taiwan University, Taiwan
1987	M.S., Institute of Public Health, College of Medicine, National Taiwan University, Taiwan
1993	Ph.D. Health Policy and Resource Management, Dept. of Epidemiology and Public Health, School of Medicine, Yale University, US.

Research and Professional Positions Held in Chronological Sequence

1987-1989	Secretary, Taipei City Government Department of Health, Taiwan
1991	Teaching assistant, Dept. of Epidemiology and Public Health, School of Medicine, Yale University, US
1991-1993	Research assistant, Dept. of Epidemiology and Public Health, School of Medicine, Yale University, US
1994	Post-doctoral research, Health Policy Research Center, National Taiwan University, Taiwan
1994-1995	Lecturer, Institute of Public Health, College of Medicine, National Taiwan University, Taiwan
1995-1998	Associate Professor, Institute of Public Health, College of Medicine, National Taiwan University, Taiwan
1998-2004	Associate Professor, Institute of Health Policy and Management, College of Public Health, National Taiwan University, Taiwan
1998-2008	Member National Health Insurance Research Database working team, National Health Research Institutes (NHRI), Taiwan
1999-2008	Consultant, Taipei City Government Department of Health, Taiwan
2004-present	Professor, Institute of Health Policy and Management, College of Public Health, National Taiwan University, Taiwan
2004-2007	Associate Editor, Taiwan Journal Public Health, Taiwan
2005-2008	Chairman, National Health Insurance Medical Expenditure Negotiation Committee, Department of Health, Executive Yuan, Taiwan
2006-2008	Professor and Director, Institute of Health Policy and Management, College of Public Health, National Taiwan University, Taiwan
2008-2009	Deputy Minister, Department of Health, Executive Yuan, Taiwan
2009-2010	Director General of National Health Insurance

2010-2016	Professor and Director, Institute of Health Policy and Management, College of Public Health, National Taiwan University, Taiwan
2013-2015	Chairman, National Health Insurance Committee, Ministry of Health and Welfare, Taiwan
2017-2019	Associate Dean, College of Public Health, National Taiwan University, Taiwan.
2018-present	Chairman, Taiwan Health Insurance Association, Taiwan.
2020-present	Dean, College of Public Health, National Taiwan University, Taiwan

Research Interests

Health Policy Analysis, Health Economics, Health System Reform

Major Honors and Awards

1996-1999	Excellent Research Award, National Science Council, Taiwan
2002	Outstanding Teacher Award, National Taiwan University, Taiwan
2008	Excellent Teacher Award, National Taiwan University, Taiwan
2010-2012	Superior Research Performance Award, National Taiwan University, Taiwan
2011	Public Health Medal Award, Department of Health, Executive Yuan
2011	Superior Society Services Award, National Taiwan University, Taiwan
2012	Excellent Teacher Award, National Taiwan University, Taiwan
2013	Excellent Teacher Award, National Taiwan University, Taiwan
2013	Outstanding Research Award, Ministry of Science and Technology, Taiwan
2014	Minister Wang Jin Naw Memorial Award for Best Paper in Health Care Management, Kimma Chang Foundation, Taiwan
2014	Excellent Teacher Award, National Taiwan University, Taiwan
2015	Excellent Teacher Award from National Taiwan University, Taiwan
2015	Research Project Excellent Award, National Health Research Institutes, Taiwan
2014-2016	Distinguished Professorship, National Taiwan University, Taiwan
2016-2017	Fulbright Scholar and Takemi Fellow, Harvard University, US
2017-2020	Superior Research Performance Award, National Taiwan University, Taiwan
2018	Excellent Teacher Award, National Taiwan University, Taiwan
2019	Excellent Teacher Award, National Taiwan University, Taiwan
2020	Excellent Teacher Award, National Taiwan University, Taiwan
2021	Outstanding Teacher Award, National Taiwan University, Taiwan
2022-25	Distinguished Professorship, National Taiwan University, Taiwan
2022	Outstanding Research Award, National Science and Technology Council, Taiwan

Investigation of Care Continuity and Care Coordination for Patients with Multiple Chronic Conditions under the Universal Health Insurance Scheme in Taiwan

Shou-Hsia Cheng[1] and Chi-Chen Chen[2]
鄭守夏[1], 陳啟禎[2]

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[2] Department of Public Health, Fu-Jen University, Taiwan

Taiwan's health care system is based on specialist and hospital care without referral requirement. Therefore, patients are free to see preferred physician for each visit. In addition, the national health insurance (NHI) program has improved the general accessibility to health care since1995. Patients in Taiwan are often criticized for their doctor-shopping behaviors. However, measuring doctor- shopping behavior is difficult. Under the single-payer NHI scheme with comprehensive claims data set, we tried to use the indicator of care continuity from a positive perspective to realize the relationship between patients and their physicians. We conducted a series of studies on the relationship between care continuity and health care outcomes. Results have revealed positive effects of care continuity on health care outcomes.

However, the growth of the elderly population with an increasing prevalence of chronic conditions have challenged the concept of care continuity. People are more likely to receive specialty care from various healthcare providers in different settings. Researchers have suggested that when patients see multiple providers, the concept of care continuity should focus not only on the ongoing interpersonal continuity between patients and their physicians but also on care coordination among multiple physicians in different care settings. Unfortunately, in Taiwan, under the high level of patient satisfaction with freedom of choice, it is difficult to improve care continuity for patients by assigning a gatekeeper or limiting their choice to visit multiple physicians.

Interestingly, the terms care coordination and care continuity are sometimes used interchangeably in the context of "fragmentation of care"; several studies have argued that distinct differences exist between the two concepts. Therefore, we examined and demonstrated the distinct and positive effects of claims-based care continuity and care coordination on health care outcome, especially for patients with chronic conditions. On the other hand, care continuity and care coordination have multidimensional concepts, claims-based care continuity and care coordination indicators is constrained by certain features. Therefore, we reviewed the concepts and measurement indicators for care continuity and coordination from the 1980s to the present and developed a patient- perspective measurement tool for continuity and coordination of care, named "Combined Outpatient Care Continuity and Coordination Assessment (COCCCA)". The findings revealed that the concepts of care continuity and coordination are distinct. Care continuity focuses on the long-term, informational, and interpersonal interaction aspects between patients and specific physicians. In contrast, care coordination primarily concentrates on the exchange of information, communication, and collaboration among different healthcare providers for the patient.

Finally, how to improve coordination among healthcare providers is still a challenge. In view of the unique feature of the single-payer insurance scheme, we proposed an "information-driven patient-centered care" model by utilizing the NHI MediClould platform to escalate the level of care coordination among physicians for their patients. We consider this is the right direction for health system reform while we are facing a fast aging population in Taiwan.

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Education

1986-1990	B.S., Occupational Therapy, Rehabilitation Medicine, National Taiwan University, Taiwan
1991-1993	M.S., Occupational Therapy, Boston University, U.S.A.
1993-1997	Sc. D., Occupational Therapy, Rehabilitation Science, Boston University, U.S.A.

Research and Professional Positions Held in Chronological Sequence

1997-2001	Assistant Professor, Department of Occupational Therapy (OT) Chang Gung University
2001-2008	Associate Professor, OT Dept. & Graduate Institute of Clinical Behavioral Science, Chang Gung University
2008-2018	Professor, OT Dept. & Graduate Institute of Clinical Behavioral Science, Chang Gung University
2008-2022	Chair, OT Dept. & Graduate Institute of Clinical Behavioral Science, Chang Gung University
2018-present	Distinguished Professor, OT Dept. & Graduate Institute of Clinical Behavioral Science, Chang Gung University
2020-present	President, Taiwan Occupational Therapy Association
2022-present	Associate Dean of College of Medicine, Chang Gung University

Research Interests

Neurorehabilitation, Stroke rehabilitation, Motor control and learning, Cognitive rehabilitation, Health promotion for the elderly, Virtual reality

Major Honors and Awards

2008-2011	Listed in the Marquis Who's Who in the World
2008-2010	Listed in 2000 OUTSTANDING INTELLECTUALS OF THE 21st CENTURY, International Biographical Centre, Cambridge, England
2009	Listed in 21st Century Award for Achievement
2009-2010	Listed in Who's Who in Medicine & Healthcare
2016-2017	Recipient of Senior Fulbright Scholar Scholarship
2018	Awarded Member of the Academy of Research at American Occupational Therapy Foundation, USA
2018	Fellow, the American Occupational Therapy Foundation Academy of Research

2019	Keynote Speaker, Designing robot-assisted training programs for upper extremity recovery of stroke patients, the 2nd Japan-Taiwan Occupational Therapy Joint Symposium, Fukuoka, Japan
	Keynote Speaker, Cutting-edge occupational therapy for motor recovery after stroke, 2019 Korea/Taiwan Joint Conference, Seoul, South Korea
2022	Invited moderator, Session: Taiwan Occupational Therapy Association (TOTA) special session: Clinical application of biomechanics in occupational therapy. 9th World Congress of Biomechanics, Taiwan.
	Invited speaker, Topic: Comparative effects of electromyography-driven robot-assisted hand training and task-oriented training on motor and daily function in patients with stroke: a randomized crossover study. 9th World Congress of Biomechanics, Taipei, Taiwan.
2023	Top 2% Scientists Worldwide 2022 by Stanford University

Non-invasive Brain Stimulation in Stroke Rehabilitation and the Application of EEG Markers

Ching-Yi Wu[1], Chia-Lun Liu[1], Chia Ling Chen[2], Kuchou Chang[3], Yu Wei Hsieh[1], Chien-Ting Liu[4], Pei-Kwei Tsay[5]

吳菁宜[1], 劉嘉倫[1], 陳嘉玲[2], 張谷州[3], 謝妤葳[1], 劉建廷[4], 蔡培癸[5]

1. Department of Occupational Therapy, Chang Gung University, Taiwan.
2. Graduate Institute of Early Intervention, Chang Gung University, Taiwan.
3. Division of Cerebrovascular Diseases, Kaohsiung Chang Gung Memorial Hospital, Taiwan.
4. Department of Physical Medicine and Rehabilitation, Dachien Hospital, Miaoli, Taiwan.
5. Department of Public Health and Parasitology, Chang Gung University, Taiwan.

Stroke remains to be a leading cause of disability. Consequently, developing effective rehabilitation interventions to maximize functional recovery is a major challenge in stroke rehabilitation. Mirror therapy (MT) is one of the promising interventions for improving sensorimotor recovery in stroke patients, as recommended by the American Heart Association. Recently, neurostimulation approaches, particularly transcranial direct current stimulation (tDCS), have also shown to be effective and easy to combine with other therapies. Therefore, tDCS is often applied in conjunction with other treatments. This prompted my team to propose a novel hybridization of tDCS and MT to further augment the benefits of MT for maximizing brain plasticity. The first part of my talk will assess the impact of hybrid tDCS-MT intervention on various clinical measures. In this study, the parameters of tDCS sites (premotor cortex, primary cortex, and sham) will be examined in relation to their effectiveness. Although this hybrid tDCS-MT intervention is effective, the underlying therapeutic mechanisms are still unclear, making further protocol modifications difficult. Electroencephalogram (EEG) is an appropriate tool for examining neural mechanisms because its relevant indexes, such as oscillatory activity, are associated with specific functional roles. the gating-by-inhibition model proposes that at the neurophysiological level, the execution of an action is accompanied by a distinct evolving spatial pattern of EEG activity in the alpha frequency band. Specifically, an increase in alpha power in the temporal lobe is associated with the suppression of non-motor activity (e.g., motor memory), while a reduction of alpha power in the central-frontal area is linked to the facilitation of motor activity. Based on this, we hypothesized that the EEG indexes of alpha power would indirectly contribute to the enhancement of motor recovery, serving as more sensitive neural indexes than clinical measures. Moreover, the relationships between alpha oscillatory activity and clinical improvement would vary according to the site of tDCS stimulation. The results indicate that the combined premotor tDCS and MT did facilitate motor recovery; however, its effect did not manifest immediately but emerged after three months. Importantly, this long-term benefit is closely associated with a change in temporal alpha. Inspired by the effectiveness of temporal alpha, we also found that individual alpha power at baseline from all intervention groups can predict long-term motor recovery. The last part of my talk will focus on the effectiveness of these EEG indexes on the recovery of activities of daily life (ADL), which often receive less research attention. As our previous studies have demonstrated the role of temporal alpha power in filtering non-motor involvement for better motor recovery, we hypothesized that inhibiting temporal activity during rehabilitation could shield the motor relearning process from unnecessary verbal/cognitive interference. The results of consistent correlations between ADL and temporal alpha, observed again in the premotor tDCS group, clearly support this possibility. In conclusion, this series of studies demonstrates the potential of the hybrid tDCS approach and the additional benefits of introducing theory-driven neural indexes in explaining underlying therapeutic mechanisms.

Hsiang-Tsui Wang

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Education

2001-2004	B.S., Pharmacy, National Taiwan University, Taiwan
2004-2006	M.S., Biochemistry and Molecular Biology, National Taiwan University, Taiwan
2008-2012	Ph.D., Environmental Medicine, New York University, USA

Research and Professional Positions Held in Chronological Sequence

2012-2014	Post-Doctor, New York University Langone Medical center, USA
2014-2020	Assistant Professor, National Yang-Ming University, Taiwan
2020-2024	Associate Professor, National Yang Ming Chiao Tung University, Taiwan
2024-present	Professor, National Yang Ming Chiao Tung University, Taiwan

Research Interests

For the past decade, my research has centered on unraveling the intricate molecular pathways through which acrolein triggers a spectrum of human ailments, spanning from cancer to metabolic and neurodegenerative disorders. Acrolein, an α,β-unsaturated aldehyde, is pervasive in both our diets and the environment, while also being generated internally through lipid peroxidation and polyamine oxidation processes. Our recent investigations underscore the pivotal role of acrolein in various pathologies, particularly under hypoxic or hyperglycemic conditions, such as cancers, diabetic kidney diseases, and neurodegeneration. Illuminating the mechanisms underpinning acrolein's involvement in these maladies holds promise for advancing early detection methods and developing targeted therapeutic interventions.

Major Honors and Awards

2018	Diplomate of the American Board of Toxicology, American Board of Toxicology
2019	Award for Junior Research Investigator, Dr. Tsung Ming Tu Foundation
2023	Teaching Excellence Awards, National Yang Ming Chiao Tung University
2023	Excellent Paper Award, Veterans General Hospitals and University System of Taiwan Joint Research Program

To Elucidate the Interplay of Aldehyde Dehydrogenase 2 and Acrolein in Chronic Kidney Diseases

Hsiang-Tsui Wang[1], Szu-yuan Li[2], Ming-Tsun Tsai[2], Chih-Ching Lin[2]
王湘翠[1]，黎思源[2]，蔡明村[2]，林志慶[2]

[1]Institute of Pharmacology, College of Medicine, National Yang Ming Chiao Tung University, Taiwan
[2]Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taiwan

Variations in the incidence of chronic kidney diseases (CKD) among different racial groups stem from a combination of genetic and environmental influences. Among East Asians, the Glu504Lys polymorphism in aldehyde dehydrogenase 2 (ALDH2) is prevalent, impairing the function of this crucial enzyme responsible for detoxifying aldehydes like acrolein. This polymorphism is linked to reduced kidney function. Our research seeks to explore the connection between changes in acrolein levels and ALDH2 in the development of kidney fibrosis. Clinical data reveals correlations between ALDH2 expression in the kidneys, estimated glomerular filtration rate, urinary acrolein levels, and the severity of kidney fibrosis. Lower levels of ALDH2 are associated with a poorer prognosis in CKD patients. In mouse models of unilateral ureteral obstruction and folic acid nephropathy, elevated levels of acrolein and decreased ALDH2 levels are observed in the kidneys, particularly in ALDH2 Glu504Lys knock-in mice. Accumulated acrolein alters the function of pyruvate kinase M2, prompting its migration from the cytosol to the nucleus in kidney tubular epithelial cells. This shift leads to changes in mitochondrial function, contributing to tubular damage and progressive kidney fibrosis. Boosting ALDH2 expression through adeno-associated virus vectors decreases acrolein levels and attenuates fibrosis in both wild-type and Glu504Lys knock-in mice. These findings propose a potential therapeutic avenue for individuals with CKD by targeting the intricate interplay between ALDH2 and acrolein.

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Education

1986-1990	B.S, Public Health, National Taiwan University, Taiwan
1990-1995	Ph.D., Epidemiology and Public Health, University of London, UK

Research and Professional Positions Held in Chronological Sequence

1995-1996	Post-doctoral Researcher, Department of Gerontology, University of Cambridge, UK
1996-1997	Post-doctoral Researcher, Institute of Biomedical Sciences, Academia Sinica, Taiwan
1996-1997	Assistant Professor, Department of Public Health, Chung-Shan Medical University, Taiwan
2000-2004	Adjunct Assist. Professor, Department of Public Health, National Cheng Kung University, Taiwan
2010-2011	Advisor, Teaching Excellence Promotion Center, Chung-Shan Medical University, Taiwan
2013-2019	Adjunct Professor, Ph.D. Program in Environmental and Occupational Medicine, Kaohsiung Med. University, Taiwan
2013–2014	Visiting Scientist, National Institute of Environmental Health Sciences, National Institutes of Health, USA
2022-2023	Department of Environmental Health Sciences, Columbia University, New York, USA
2010-present	Adjunct Professor, Department of Public Health, China Medical University
2014-present	Research Center for Environmental Medicine, Kaohsiung Medical University, Taiwan
2015-present	Adjunct Professor, School of Public Health, National Defense Medical Center, Taiwan
2016-present	Adjunct Professor, Department of Safety, Health and Environmental Engineering, National Union University, Taiwan
2009-present	Full Investigator, National Institute of Environmental Health Sciences, NHRI, Taiwan

Research Interests

My research is mainly on Children and Maternal Environmental Health, particularly in human developmental stages. During the time, fetus, young children, or fertility women are vulnerable, and early preventions are crucial for life-long health and cost effectiveness.

I will continue to explore important environmental factors that affect human health, not only the impact of single pollutant exposure, but also the impact of multiple environmental pollutant

exposures or environmental physiology on human health. Besides, the interactions of these factors with genes, especially in developmentally sensitive periods of life such as early childhood and adolescence, will be discussed in the future. The aim is to propose preventive suggestions and treatment strategies, building upon the concepts outlined in the following points: 1. 'Fetal Origin of Health and Disease' theory; 2. Applying research results to the prevention of adult chronic diseases; 3. Understanding and addressing the impact, prevention, and treatment of individuals in highly exposed areas to help safeguard the health of the next generation.

By exploring how the environment affects gene expression and how genes and epigenes modify the effects of toxicants, we can develop detoxification strategies for environmental hazards (e.g., folic acid supplementation) and promote the development of healthy foods (e.g., probiotics) that mitigate their effects. Ultimately, the goal is to achieve disease prevention and health promotion through scientific understanding and practical application.

Major Honors and Awards

1995	International academic travel award, European Association of Study in Diabetes (EASD)
1996	International travel award, International Diabetes Federation (IDF)
1997	In international academic conference award, Academia Sinica
2002	Young Scientist Award, Asian Conference Occupational Health Meeting (ACOH)
2006	Young Scientist Award, National Health Research Institutes, Taiwan
2009-2010	**Who's Who** in Medicine and Healthcare
2015-2016	Proposal Reexamination advisory committee member of Department of Natural Sciences and Sustainable Development (Division of Sustainability), Ministry of Science and Technology (**MOST**), Taiwan.
2016-2019	Proposal Reexamination **advisory committee member** of Department of Life Science (Division of Social Medicine and Division of Mother and Child Medicine), Ministry of Science and Technology (MOST), Taiwan.
2017-2018	Secretary General of ISEE-AC
2018	Conference **co-organizer of ISEE-AC** (Asia Chapter) Biennial meeting in Taiwan with Director Guo YL and Dean Chan CC
2019-2020	**Chair Elect** of the ISEE - AWPC
2020-2023	**Chair of the ISEE** (International Society for Environmental Epidemiology) - AWPC (Asia and Western Pacific Chapter)
2021-2024	Proposal Reexamination **advisory committee member** of Division of Environmental Medicine and Public Health, National Science and Technology Council (**NSTC**), Taiwan.
2024	2024 National Health Research Institutes Research Achievement Award - **Outstanding Research Achievement Award**

Developmental Origin of Atherosclerosis and Neurocognitive Dysfunctions from Environmental Endocrine Disruptors

Shu-Li Wang
王淑麗

National Institutes of Environmental Health Sciences, National Health Research Institutes, Taiwan

Shu-Li and her team have utilized the birth cohorts established in 2000-01 and 2012-14 to investigate the long-term impact of environmental factors, such as heavy metals and plasticizers, on neurocognitive and immune health during different growth stages involving epigenetic changes.

Specifically, maternal exposure to inorganic arsenic has been linked to neonatal gene methylation and an increased risk of vascular disease. Early inorganic arsenic intake can raise low-density lipoprotein concentration and atherosclerosis index in children during adolescence. Prenatal exposure to inorganic arsenic may also increase children's risk of asthma, allergic rhinitis, and atopic dermatitis. Furthermore, central nervous system development and cognitive behavior are susceptible to the effects of environmental endocrine-disrupting chemicals during the fetal development period.

Moreover, maternal exposure to high concentrations of phthalate plasticizers affects sex hormone regulation in pregnant women, newborns, and children and is associated with subsequent reproductive organ and pubertal development. Encouragingly, the methylation of some genes (PA2G4, HMGCR, XRCC6) in fetal umbilical cord blood changes with exposure to DEHP plasticizer during pregnancy. These genes are related to estrogen and androgen response, sperm production, and other functions.

In summary, the findings from this research underscore the importance of early preventive measures for chemical substance management. They also highlight the potential health risks associated with environmental factors during different growth stages. Future work will focus on evaluating psychological and immune diseases in adulthood in relation to environmental and physiological changes in early life, providing further insights into the long-term health effects of early environmental exposures.

Poster List

Group A

Group B

Group C

Group D

Group E

Title of Project: Prohibitin 2-mediated Mitophagy in Organismal Healthspan and Lifespan
Project No.: NHRI-EX113-11009SC
P.I. Name: Wei-Chung Chiang/姜為中
Key Professional Personnel: Yu-Shan Lin/林煜珊, Pei-Jing Lee/李沛靖, Pei-Han Liu/劉沛函
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2021 to 2024 (Total: 4 years)

The selective degradation of damaged or unwanted mitochondria via autophagy (mitophagy) is a mitochondrial quality control mechanism postulated to play key roles in cellular homeostasis, development, metabolism, and protection against aging and age-related disorders. Our previous study identified a key mechanism of mitophagy in which a mitochondrial inner membrane protein (IMM), prohibitin 2 (PHB2), functions as a mitophagy receptor that targets the damaged mitochondria for autophagic degradation. Upon mitochondrial damage, the dissipation of mitochondrial membrane potential results in proteasome-dependent rupture of the mitochondrial outer membrane (OMM) that allows cytoplasmic exposure of PHB2 for a direct LC3 (autophagosomal membrane protein) interaction and subsequent autophagic removal.

As mitophagy is postulated to play a pivotal role in longevity, the protection against neurodegenerative diseases and metabolic disorders, we aimed to (1) create a specific mouse genetic model of defective PHB2-mediated mitophagy and use this tool to examine the role of mitophagy in organismal health; (2) identify novel autophagic molecules that “sense” the OMM rupture; and (3) understand the mechanisms that drive OMM rupture during mitophagy. Overall, these studies revealed the role of mitophagy in specifying organismal health and broadened our understanding of the role of OMM rupture in selective autophagy.

Title of Project: Metabolomic Alleviation of Osteoporosis: Lipidomic Control of Epigenetic Action to Stem Cell Program

Project No.: NHRI-EX113-11029SI

P.I. Name: Feng-Sheng Wang/王逢興

Key Professional Personnel: Feng-Sheng Wang/王逢興, We-Shiung Lian/連韋雄, Jih-Yang Ko/郭繼陽, Ming-Hong Tai/戴明泓, Yu-Shan Chen/陳于珊, Shao-Yu Wang/王紹諭

Affiliation/Institution: Chang Gung Medical Foundation

Entire Project Period: From 2021 to 2027 (Total: 3 years)

Background: Dysregulated bone-making capacity accelerates the development of osteoporosis, which turns well-woven bone network into low bone mass and porous microarchitecture putting aged people in a risk of skeleton fracture-associated disability. Epigenetic histone methylation and metabolomic alterations are correlated with bone mass homeostasis and osteoporosis development. Little is known about the role metabolites may play in bone integrity regulated by histone demethylase.

Objectives: This study was aimed to utilize osteoblast-specific histone H3K27me3 demethylase Utx conditional knockout mice (Utx-KO) and short-chain fatty acid receptor GPR43 transgenic mice (GPR43Tg) and characterized metabolomic landscapes of osteoblasts during osteoporosis development. We also investigated whether *in vivo* manipulation of TCA cycle intermediate changed UtxKO or estrogen deficiency-mediated osteoporosis development.

Materials and Methods: Bone mineral density, trabecular bone microstructure, and body adipose in UtxKO mice or UtxKO-GPR43Tg mice were characterized using μCT imaging. Metabolomic landscapes of osteogenic cells were investigated using capillary electrophoresis time of flight mass spectrometry (CE-TOFMS). UtxKO mice or ovariectomized mice were fed TCA cycle intermediates a-ketoglutarate (a-KG) or succinate in drinking water (pH 7.2) ad libitum. Osteogenic differentiation and adipocyte formation of bone-marrow mesenchymal cells were investigated using von Kossa stain and fluorescence Nile red stain, respectively.

Results: UtxKO mice developed severe osteoporotic skeleton together with decreased short-chain fatty acid receptor GPR43 signaling in osteoblasts. Loss of Utx function affected metabolomic profiles, which may contribute to glycolysis, TCA cycle, b-oxidation, and glutamate metabolism. Impaired a-KG-succinate metabolism process was, among others, functional pathway regulated by Utx. Specifically, a-KG treatment reversed H3K27 trimethylation, glycolytic/mitochondrial energetic programming, reducing adipocyte formation to preserved osteogenic differentiation capacity of bone-marrow mesenchymal progenitor cells in UtxKO mice. Likewise, a-KG supplementation slowed the development of osteoporotic bone in UtxKO mice and in ovariectomized mice. Osteoblast-specific GPR43 transgenic mice showed relatively high bone mass, trabecular/cortical bone microarchitecture, and mechanical strength. Gain of GPR43 function in osteoblasts counteracted bone mass loss in UtxKO mice and in ovariectomized mice.

Conclusions: Our data conveys productive insight into the bone-protective actions of TCA cycle intermediate and short-chain fatty acid signaling to cellular energy metabolism and epigenetic histone methylation in osteogenic cells regulated by Utx. These investigations also highlight new metabolite options for keeping bone tissue away from osteoporosis development.

Title of Project: TERRA RNA in the Regulation of Cellular Senescence and Ageing
Project No.: NHRI-EX113-11107SI
P.I. Name: Hsueh-Ping Chu/朱雪萍
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Telomere length becomes shorter with age, and is believed to be a predictor of longevity in human. TElomeric Repeat containing RNA (TERRA) is composed of UUAGGG repeats and transcribed from subtelomeric regions toward telomeric ends. We have recently reported that TERRA is associated with telomerase complex, and is involved in controlling telomere integrity in mammalian cells. TERRA forms DNA:RNA hybrids (R-loops) at telomeres that lead to DNA damage response and replication stress. However, how TERRA participates in the ageing process in humans is largely unknown. Utilizing TERRA-capture RNA-seq and Oxford Nanopore direct RNA sequencing to acquire full-length TERRA, we annotate TERRA transcription regions in the human T2T-CHM13 reference genome. TERRA transcripts encompass hundreds to over a thousand nucleotides of telomeric repeats, predominantly originating from 61-29-37 bp repeat promoters enriched with H3K4me3, RNA pol II, CTCF, and R-loops. We develop a bioinformatics tool, TERRA-QUANT, for quantifying chromosome-end-specific TERRA using RNA-seq datasets and find that TERRA increases with age in blood, brain, and fibroblasts. TERRA upregulation in aged leukocytes is confirmed by RT-qPCR. Additionally, abnormal TERRA expression is observed in patients with Hutchinson-Gilford progeria syndrome. Single-cell RNA-seq analysis demonstrates TERRA expression across various cell types, displaying elevated TERRA in neurons during the early stage of Alzheimer's disease. Our study reveals the association of TERRA with human aging and diseases.

Title of Project: Using Novel Endo-lysosomal Patch-clamp to Investigate the Mechanism of TPC2 and TRPML2 in Viral Trafficking and Its Implication in the Viral Diseases

Project No.: NHRI-EX113-11119SC
P.I. Name: Cheng-Chang Chen/陳政彰
Key Professional Personnel: Cheng-Chang Chen/陳政彰
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Whole cell patch-clamping and electrophysiological techniques are considered the gold standard for drug screening in both pharmaceutical research and academia. However, these methodologies are predominantly limited to characterizing ion channels on the plasma membrane, thereby neglecting the dynamic intracellular organelle systems, particularly endolysosomes. Given the critical involvement of the endolysosomal system in neurodegenerative diseases, metabolism, and infectious diseases, there is a pressing need to develop techniques that enable the direct observation of endolysosomal ion channels for advanced drug discovery. To bridge this gap, we have developed a novel whole-endolysosomal patch-clamp technique that allows for the direct electrophysiological characterization of endolysosomal ion channels. Our research has provided the first direct evidence demonstrating that TRPML2 channels conduct essential cations, which are crucial for the primary immune response against pathogens. Our study identifies a pivotal role for TRPML2 in cellular defense mechanisms against Salmonella infection. We have demonstrated that TRPML2-mediated Mg^{2+} permeability exerts an immunological effect on intracellular bacteria, thereby elucidating a key aspect of the anti-infection process. These findings contribute significantly to the conceptual framework of how TRPML2 facilitates cellular defense against pathogen invasion. In the context of emerging infectious diseases, there is an urgent necessity for precise and preventative medical interventions. Understanding the intricate mechanisms of cellular immunity, specifically the role of the endolysosomal system during pathogen infection, is critical. Our innovative intracellular organelle patch-clamping has revealed that TRPML2 is a highly active cation channel in monocyte phagocytic cells. Through the development of small-molecule compounds targeting this ion channel, we have elucidated essential function of TRPML2 in the recycling of endosomes, which is integral to innate immunity. Employing TRPML2-/- human monocytes, recycling endosome patch-clamp techniques, calcium imaging, and cellular trafficking assays, we have demonstrated that the loss of TRPML2 function compromises immune defense by disrupting the accumulation of endosomes at sites of fungal invasion. Furthermore, the use of TRPML2-specific agonists underscores their potential in modulating TRPML2 activity during early pathogen infection. Our results provide a detailed mechanistic understanding of how TRPML2 activation, regulated by phosphoinositides and Rab proteins, controls endolysosomal positioning during infection, thereby highlighting its therapeutic potential in enhancing immune responses. Notably, activation of TRPML2 is required for defense against fungal invasion but not for SARS-CoV-2 infection. This research underscores the potential of TRPML2 as a target for therapeutic interventions aimed at bolstering host immune responses, offering significant implications for the treatment of infectious diseases.

Title of Project: Investigate the Pathogenesis and Environmental Fitness of *Listeria monocytogenes* Emerging Clone SL87

Project No.: NHRI-EX113-11120SC

P.I. Name: Yu-Huan Tsai/蔡雨寰
Key Professional Personnel: Dai-Ling Chang/張岱玲, Wen-Yen Weng/翁文彥
Affiliation/Institution: National Yang Ming Chiao Tung University

Entire Project Period: From 2022 to 2025 (Total: 4 years)

Human listeriosis is a foodborne disease caused by *Listeria monocytogenes* (*Lm*), and presents life-threatening illness mainly characterized by bloodstream infection, maternofetal or neonatal infection, and meningoencephalitis as central nervous system infection. By collaborating with Taiwan CDC, we performed a nationwide study between 2014 and 2019 of both clinical and food *Lm* isolates and sequenced their genomes, in which in 2018 and 2019 the clinical isolates were collected in a mandatory manner in Taiwan. We found that SL87 (37.1%), SL5 and SL378 accounted for the majority (65%) of clinical cases. Unexpectedly, SL87 and SL378 were also predominant (57%) in food products. These findings indicate that, in contrast to the *Lm* in France, the *Lm* clones in Taiwan may possess both pathogenic potency and environmental persistence. In this study, we aim to study environmental persistence and pathogenicity of the major SLs prevalent in Taiwan, focusing on SL87 due to its high prevalence in clinical infection. We found that SL87 produced more biofilms than SL378 at 37 ˚C. In contrast, SL378 had better biofilm formation capacity than SL87 at 4 ˚C. These results suggest that the *Lm* clones prevalent in Taiwan have distinct surface persistence capacity in a temperature-dependent manner. Using human monocytic cell infection platform, we found that SL87 has the highest intracellular bacterial load among the SLs irrespective of the isolation source. Comparative genomics identified the presence of LIPI-4, a pathogenic island contributes to *Lm* invasion into central nervous system and placenta in SL4, in all the SL87 isolates but not in other isolates in Taiwan. We established a platform to efficiently knock out genes in SL87 without leaving any selection marker. Deletion of LIPI-4 in three different cgMLSTs of SL87 isolates modulated expression of *actA*, which encodes ActA for *Lm* cell-to-cell spread and biofilm formation. Future work will focus on how LIPI-4 regulates the expression of ActA in *Lm*.

Title of Project: Analysis of Mitochondria and Nucleus Communication in Retrograde Signaling in Cardiac Cells

Project No.: NHRI-EX113-11121SC

P.I. Name: An-Chi Wei/魏安祺

Key Professional Personnel: An-Chi Wei\魏安祺, Yi-Ju Lee/李羿儒

Affiliation/Institution: National Taiwan University

Entire Project Period: From 2022 to 2025 (Total: 4 years)

When mitochondria in cardiac myocytes experience stress, damage, or dysfunction, mitochondrial retrograde signaling is activated to coordinate adaptive responses and maintain cellular homeostasis. This signaling pathway regulates cellular processes such as metabolism, antioxidant defenses, and removing damaged mitochondria through mitophagy. In this study, we used a mitochondrial toxin doxorubicin (DOX) to induce stress to study the relationship between mitochondrial retrograde signaling and cardiomyopathy. Doxorubicin is an anticancer drug that inhibits topoisomerase II. It is also known to cause severe acute or chronic mitochondrial-related cardiotoxic side effects through the excessive generation of reactive oxygen species, inhibition of the electron transport chain, and destruction of mitochondrial DNA. Cultured AC16 human cardiomyocytes were treated with 0.1 μM DOX for 2, 4, or 6 days. Following treatment, the cell viability, mitochondrial function, and mitochondrial protein levels were analyzed. RNA sequencing analysis and quantitative metabolomic analysis were performed to identify differences caused by DOX. The results show reduced cell viability in DOX-treated cells; however, the cell size increased up to 5 to 10 fold. The upregulation of oxidative phosphorylation complexes and increased glucose uptake were observed in surviving cells after DOX treatment. Furthermore, through pathway analyses of RNA-seq data, doxorubicin activated the p53, MAPK, NF-κB, Erk1/2, and IL-6 signaling pathways, possibly accounting for the hypertrophy observed in surviving cells after DOX treatment. These findings suggest that the mitochondrial retrograde signaling pathway is activated, initiating the compensatory effect of doxorubicin toxicity and providing further insights into the underlying mechanisms of DOX-induced cardiotoxicity.

Title of Project: Role of Endothelial ER Protein TXNDC5 in Pulmonary Arterial Hypertension: Mechanistical Insights into Endothelial-mesenchymal Transition

Project No.: NHRI-EX113-11138SI

P.I. Name: Wei Ting Chang/張瑋婷
Key Professional Personnel: Yu-Wen Lin/林育雯, Ying-Gen Tsai/蔡英菁
Affiliation/Institution: Chi Mei Medical Center

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Right ventricular (RV) failure is a major cause of morbidity and mortality in pulmonary arterial hypertension (PAH), but its mechanism remains largely unknown. Recently studies have found that endothelial to mesenchymal transition (EndMT) is known to be associated with PAH pathogenesis. Thioredoxin domain containing 5 (TXNDC5), an ER-resident protein, is involved in pulmonary fibrosis, but its effects on pulmonary arterial remodeling require investigation. Herein, we studied the regulatory mechanism of TXNDC5 by promoting EndMT in pulmonary artery remodeling and subsequent RV failure.

In our previous work, immunofluorescence staining revealed marked TXNDC5 upregulation in vimentin^{+} fibroblast cells in PAH patients, suggesting its role in lung fibrosis. Western blot assay showed increased levels of TXNDC5, vimentin, HIF-1α, and Twist1, along with decreased VE-Cadherin, in pulmonary artery (PA) tissues of PAH patients compared to healthy controls. In the Sugen/hypoxia-induced PAH mouse model, PAH increased TXNDC5 production and triggered EndMT in PA remodeling, leading to RV failure. However, these phenomena were reversed in endothelial-specific TXNDC5 knockout mice (Txndc5^{cKO}). In the 3rd year, we demonstrated that TXNDC5-regulated EndMT progression was essential for hypoxia-inducible factor-1 (HIF-1) responses in human pulmonary microvascular endothelial cells (HPMECs) under hypoxic conditions. HIF-1, a heterodimeric transcription factor, consists of two subunits: HIF-1α and HIF-1β (ARNT). Inhibiting HIF-1α expression with 2ME2 suppressed TXNDC5 levels in HPMECs under hypoxia. Conversely, inhibiting TXNDC5 led to downregulation of both HIF-1α and HIF-1β transcription. Promoter assays confirmed that HIF-1β signaling induces TXNDC5 transcription. Additionally, SMAD3, a key player in fibrosis, was downregulated following TXNDC5 siRNA treatment. Further promoter assays showed that deleting the SMAD3 binding site significantly reduced HIF-1β promoter activity in response to hypoxia or $CoCl_2$ stimuli.

These findings indicate that hypoxia induces HIF-1α, which recruits HIF-1β to form a complex, increasing TXNDC5 expression. TXNDC5 then regulates HIF-1β levels via the SMAD3 signaling pathway. Targeting TXNDC5 with Aptamers in rodent models of PAH improved RV function and reduced pulmonary artery and cardiac fibrosis. In conclusion, PAH increases TXNDC5 production, triggering EndMT in PA remodeling and leading to RV failure. Thus, targeting TXNDC5 could be a promising therapeutic approach to reduce lung fibrosis and improve cardiac function in PAH patients.

Title of Project: The Fate and Role of Kidney Pericytes During Acute Kidney Injury - Chronic Kidney Disease Continuum

Project No.: NHRI-EX113-11139SI
P.I. Name: Shuei-Liong Lin/林水龍
Key Professional Personnel: Yu-Hsiang Chou/周鈺翔, Yu-Han Shao/邵鈺涵, Man-Tzu Li/李曼慈, An-Jie Luo/駱諳潔
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Acute kidney injury (AKI) is an important disease or complication with high mortality. In addition, more and more studies found that high proportion of AKI patients develop chronic kidney disease (CKD) and progress to end-stage renal disease (ESRD) during follow-up in outpatient clinics even though they survive to be discharged from hospital without significant impairment of kidney function. Therefore, subclinical AKI might be a critical piece of the CKD unknown etiology puzzle. In patients with glomerulonephritis with AKI, immunosuppressive therapy may be effective to improve kidney function. But in patients with AKI due to the other etiologies, most of the clinically available treatments, including acute dialysis, are supportive to wait for the spontaneous repair and regeneration of kidneys after injury. No treatment of proven efficacy can be used clinically to promote the repair and regeneration. Our previous studies have proven the role of pericytes in renal fibrosis, scaring, and atrophy of mice with progressive kidney disease. We recently proved that pericytes are activated and proliferate in the kidneys of mice after AKI. In the kidneys with functional recovery, ~20% of the pericytes remain and retain the higher potential for cell proliferation and scar formation, lose the function for microvascular stabilization, and promote CKD progression thereafter. Mechanistically, transforming growth factor-β1 decreases the binding of YBX2 to the promoter of *Acta2* and induces *Ybx2* hypermethylation, thereby increasing α-smooth muscle actin (αSMA) expression in pericytes. Because the activated pericytes increase in cell numbers and enclose the injured renal tubules in the acute phase with 7 days after AKI, and undergo apoptosis during renal recovery, we hypothesize that activated pericytes might promote renal recovery through stabilizing the structure and promoting the repair/regeneration of the injured tubular epithelial cells. In previous 2 years, we used the murine model of AKI to study the role of kidney pericytes in the repair and regeneration of injured tubular epithelial cells after AKI. After administration of anti-platelet-derived growth factor receptor-β (PDGFRβ) antibody in mice with AKI induced by ischemia-reperfusion injury (IRI), the recovery of renal function assessed by the plasma levels of blood urea nitrogen (BUN) and creatinine was delayed. Tissue examination showed decreased cell numbers of αSMA^+ myofibroblasts in the injured kidneys after anti-PDGFRβ antibody treatment. Interestingly, the decrease in cell numbers of αSMA^+ myofibroblasts was accompanied with the decrease in cell proliferation of tubular epithelial cells but increased cell numbers of $F4/80^+$ macrophages in the injured kidneys. Because the expression of CXCL12, also known as stroma cell-derived factor-1, was decreased in mice with anti-PDGFRβ treatment, we selectively knocked out CXCL12 in pericytes of $Gli1^{CreERT2/+};Cxcl12^{F/F}$ mice by tamoxifen administration and used littermate $Cxcl12^{F/F}$ mice as control in this year. In $Gli1^{CreERT2/+};Cxcl12^{F/F}$ mice with pericyte-specific CXCL12 knockout, the recovery of renal function from day 2 to day 7 after AKI, regeneration of tubular epithelial cells, and density of microvasculature were decreased, suggesting the critical role of pericyte-derived CXCL12 in the renal recovery from AKI. Exogenous administration of CXCL12 recombinant protein at day 3 after IRI-AKI was found to increase renal functional recovery by increasing tubular epithelial cell proliferation. The mechanism underlying the protective effect is under study.

Title of Project: To Explore the Impact of Maternal Immune System on Infantile Vasculitis and Allergic Diseases

Project No.: NHRI-EX113-11140SI

P.I. Name: Tai-Ming Ko/柯泰名

Key Professional Personnel: Jan Vincent B Beltran/簡文森, Fang-Ping Lin/林芳平, Chaw-Liang Chang/張兆良, Hsin-Hua Chen/陳信華, Wen-Cheng Chao/趙文震, Ching-Heng Lin/林敬恆, Tzu-Hung Hsiao/蕭自宏, Jing-Rong Wang/王瀞瑢

Affiliation/Institution: National Yang Ming Chiao Tung University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Kawasaki disease (KD) and multisystem inflammatory syndrome in children (MIS-C) share similar clinical manifestations, including cardiovascular complications, suggesting common underlying immunopathogenic processes. Aberrant neutrophil activation may play a crucial role in the shared pathologies of KD and MIS-C, but the associated pathogenic mechanisms and molecular drivers remain unclear. We performed a single-cell meta-analysis of neutrophil activation using transcriptomic data from 103 pediatric peripheral blood mononuclear cell (PBMC) samples across nine cohorts, including healthy controls (HC), KD, MIS-C, dengue virus infection (DNV), juvenile idiopathic arthritis (JIA), and pediatric celiac disease (PCD). Computational analyses were employed to investigate shared neutrophil transcriptional programs of KD and MIS-C linked to systemic damage and cardiac pathologies, and to suggest FDA-approved drugs for potential treatment. Our meta-analysis included 521,950 high-quality cells. Blood signatures associated with cardiovascular risks were enriched in neutrophils from KD and MIS-C patients. We identified an expansion of $CD177^{+}$ neutrophils with hyperactivated effector functions in both KD and MIS-C, but not in HC or other pediatric diseases. $CD177^{+}$ neutrophils in KD and MIS-C exhibited highly similar transcriptomes, marked by conserved signatures and pathways related to molecular damage. A shared neutrophil expression program (SNEP), potentially regulated by SPI1, was induced, conferring enhanced effector functions, especially neutrophil degranulation. Expression of CD177 and SNEP was associated with acute disease stages and attenuated during KD IVIG treatment and MIS-C recovery. Network analysis identified hub genes correlating with $CD177^{+}$ neutrophil activation. Disease-gene association analysis linked the $CD177^{+}$ neutrophil SNEP with coronary and myocardial disorders. Lastly, we identified and validated TSPO and S100A12 as main molecular targets, for which FDA-approved drugs such as methotrexate, zaleplon, metronidazole, lorazepam, clonazepam, temazepam, and zolpidem are primary candidates for drug repurposing. Our findings indicate that $CD177^{+}$ neutrophils may contribute to systemic pathological damage in KD and MIS-C. We uncovered potential regulatory drivers of $CD177^{+}$ neutrophil hyperactivation and pathogenicity, which may be targeted as a unified therapeutic strategy for both KD and MIS-C.

Title of Project: Precision Medicine for Rare Cardiac Disease: Diagnosis and Treatment
Project No.: NHRI-EX113-11141SI
P.I. Name: Wen-Pin Chen/陳文彬
Key Professional Personnel: Wen-Pin Chen/陳文彬
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

In this third-year-project, we achieve the research goal to characterize the multi-omics of human cardiomyocytes derived from LVNC proband's hiPSC (LVNC-hCM) with good drug response to simvastatin for the clarification of the rescued signaling pathways linking to the associated functional improvement. In addition to the elucidation of pathological signaling pathways leading to the abnormal cardiac development & contractile dysfunction, the results also highlight the association of metabolic dysfunction with the pathological feature of LVNC.

Multi-omics studies including the analysis of transcriptome (by RNA-seq), proteome (by LC-MS/MS) and metabolome (by CE-TOFMS & LC-TOFMS) were conducted to comprehensively characterize the altered molecular profile of LVNC-hCMs with or without drug treatment as well as in comparison to that of health-hCM derived from iPSC of heath donor (the proband's mother). The 2D annotation enrichment method was employed to analyze the quantitative data from both the proteome and transcriptome. Simvastatin can recover the expressions of cardiac molecules related to muscle contraction and mitochondrial metabolism in LVNC-hCM. The functional protein association network of the altered molecules was constructed using STRING with GOBP functional annotation. Simvastatin can restore the expressions of EZH2 (a catalytic subunit of polycomb repressive complex 2 (PRC2)), TGFβ-signaling molecules, and cardiac genes associated with cardiac contraction and metabolic processes.

Cardiac mitochondrial function in either OCR (OXOPHOS) and ECAR (glycolysis) was significantly decreased in LVNC-iPSC-hCM derived from carrying different LVNC patients carrying different mutations including TNNT2, DMD & TAFAZZIN in comparison to that in health-iPSC-hCM by sea horse assay. A decrease of H3K27Ac marks in the promoter region of the genes associated with metabolic function was detected by ChIP-seq. Simvastatin can increase malate/aspartate shuttle via metabolomic analysis. It was further demonstrated that simvastatin can improve cardiac glucose metabolic rate by PET in parallel with the increase of LVEF.

This study successfully elucidated the role of intranuclear TNNT2 in cardiac epigenetic homeostasis, the underlying mechanism of TNNT2(R141W) in inducing LVNC pathogenesis through perturbing myocyte intranuclear HDAC1 distribution and consequently leading to the dysregulation of cardiac functional genes related to cardiac development, muscular contraction and metabolism. Simvastatin can restore TNNT2(R141W)-mediated pathological signaling via the recovery of TNNT2(R141W)-HDAC1 association in myocyte nuclei.

The details of this progress report are presented in the appended document.

Title of Project: Discovery of Specific Paratopes for Anti-galactose-deficient IgA1 Autoantibodies That Induce IgA Nephropathy

Project No.: NHRI-EX113-11142SI
P.I. Name: Ann Chen/陳安
NHRI Researcher: Hong-Hsing Liu/劉鴻興
Key Professional Personnel: Chen Ann/陳安, Yu-Ching Lee/李雨青, Chia-Chao Wu/吳家兆, Shuk-Man Ka/賈淑敏, I-Lin Tsai/蔡伊琳
Affiliation/Institution: National Defense Medical Center
Entire Project Period: From 2022 to 2024 (Total: 3 years)

The pathophysiological mechanisms of IgA nephropathy (IgAN) remain largely unknown to date, while elevated serum levels of the galactose-deficient IgA1 (Gd-IgA1) autoantigens (auto-Ags) and the corresponding autoantibodies (auto-Abs) are found to form IgA1-IgG immune complexes and further localize in the affected glomeruli, initiating the development or progression of the autoimmune renal disease. The goal of our proposal is to investigate the paratopes of IgAN patient-derived auto-Abs and determine their role in the pathogenesis underlying the renal disease. **We have successfully isolated 29 such auto-Abs utilizing both human-hybridoma technology and phage-display system over the past two and half years.** Each of these auto-Abs has been studied about its ELISA binding properties, glycan array recognition, complex formation, kidney deposition, inflammation activation, gene analysis, and protein structures. Our current progress report on the new results shows the following: **[1]** Two IgG auto-Abs and one Gd-IgA1 were identified from the same IgAN patient forming a heterotrimeric immune complex with differential effects in inflammatory response. **[2]** A more in-depth analysis of gene sequences was performed, including the newly identified ones since last report, revealing that the VJ-4 class dominates these antibodies, while VH and VD have a more diverse profile. The overall mutation rate is high, especially in the FR3 region, The length of HCDR3 was also found to be shorter than that of healthy control, and the aromatic-residue-rich nature of HCDR3 could implicate a potential recognition with GalNAc. **[3]** Two scFv auto-Abs, scFv-G1 and scFv-F10, contain N-glycosylation sites in the heavy-chain CDR2; and one hybridoma auto-Ab even carries two N-glycosylation sites in the heavy-chain FR3 of heavy chains. The presence of N-glycans in the CDR inspired further tests to understand their interplay with GalNAc on the ligand. **[4]** Datasets of next-generating sequencing were performed for IgAN patients, and further analysis and comparison with healthy controls are underway. **[5]** Using virtual docking annotation/algorithm to unveil both the Fab sequences (molecular signatures) and specific glycoforms of Gd-IgA1 hinge region (bearing Tn) revealed a vast majority (more than 90%) of the molecular dynamic conformations maintaining rigid linker between Fab–Fc, while galactose-deficiency on key positions led to flexible conformations and antigenic epitope exposure. **[6]** A pretrained antibody language (AI) model was established to encode the autoantibody and relatively normal IgG sequences for visualization and classification. **[7]** Identification of new IgG auto-Abs from human hybridoma cell lines and phage-display platform is still ongoing by our Taiwan Autoantibody Biobank Initiative (TABI) in Hualien Tzu Chi Hospital.

Title of Project: Targeting the Ketogenesis Pathway for Tissue Rejuvenation
Project No.: NHRI-EX113-11203SI
P.I. Name: Patrick Ching-Ho Hsieh/謝清河
Key Professional Personnel: Yi-Chan Lee/李亦展
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2023 to 2027 (Total: 5 years)

Introduction Cardiac ketogenesis regulation of metabolism is beneficial in promoting adult cardiomyocyte dedifferentiation and proliferation after myocardial infarction, which can improve recovery time and overall prognosis (Cheng et al. Circulation, 2022). A ketogenic diet is also shown to prolong the lifespan of healthy aged mice (Roberts et al. Cell Metabolism, 2017), however, the influence of cardiac ketogenesis in regulating cardiac aging remains unknown.

Hypothesis If cardiac ketogenesis prevents cardiac aging, then loss of ketogenesis key enzyme HMGCS2 will result in metabolic changes and heart dysfunction leading to premature cardiac aging.

Methods and Results To investigate the impact of disrupted ketogenesis in the heart, cardiomyocyte-HMGCS2 KO mice were generated by deleting sites flanking exon 2 of HMGCS2 through cross-breeding with α-myosin heavy chain promoter-driven (αMHC) CRE mice. Cardiomyocyte-HMGCS2 KO mice showed a lower survival rate during the aging process compared with age-matched control mice. Additionally, the echocardiographic analysis revealed defective heart functions including decreased left ventricle ejection fraction and stroke volume, and increased myocardial performance index and left ventricular diastolic time constant (Tau) in cardiomyocyte-HMGCS2 KO hearts. On histological slides, cardiomyocyte-HMGCS2 KO hearts displayed a higher fibrosis ratio, enlarged cardiomyocyte cell size, and injured mitochondrial structure. Cardiomyocyte-HMGCS2 KO hearts also displayed reduced oxidative phosphorylation complex protein expression and oxygen consumption rate in mitochondria, indicating mitochondrial impairment. Metabolic composition and adaptation of hearts analyzed by C^{13}-labeled metabolites and nuclear magnetic resonance revealed reduced tricarboxylic acid cycle-associated metabolites and oxidative adoption in cardiomyocyte-HMGCS2 KO hearts.

Conclusion These results suggest that loss of HMGCS2 in cardiomyocytes accelerates cardiac aging through cardiac and mitochondrial dysfunction.

Title of Project: Modeling Rare Cardiac Disease of Polyglucosan Body Myopathy1 and Exploring Its Underlying Molecular Mechanisms
Project No.: NHRI-EX113-11232SI
P.I. Name: Su-Yi Tsai/蔡素宜
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Polyglucosan body myopathy 1 (PGBM1) is a rare glycogen storage disease (GSD) caused by a deficiency in the ubiquitin ligase RBCK1. This deficiency leads to the accumulation of polyglucosan bodies in muscle tissues, resulting in early-onset muscle weakness and dilated cardiomyopathy (DCM). While RBCK1's role in immunity is known, its impact on glycogen metabolism has been unclear.

Our study used human pluripotent stem cells to investigate RBCK1's role in PGBM1. We found that RBCK1 deficiency causes polyglucosan body accumulation and DCM-like symptoms, including metabolic dysfunction and abnormal calcium handling. Crucially, we identified a mitochondrial regulator as an interactor with RBCK1. The deletion of RBCK1 significantly reduced this regulator's expression, causing a key metabolic enzyme to mislocalize to the cytoplasm, disrupting glycogen metabolism. These findings suggest that RBCK1-related PGBM1 involves the mitochondrial regulator's effect on the metabolic enzyme, providing new therapeutic insights for PGBM1 patients and potential targets for intervention.

Title of Project: Imbalanced Gut-lung Axis in Developing Susceptibility to Nontuberculous Mycobacterial Lung Disease: Focusing on the Immune Mechanisms and the Effect of Restoration in Gut Microbiota

Project No.: NHRI-EX113-11233SI
P.I. Name: Chin-Chung Shu/樹金忠
Key Professional Personnel: Hsin-Chih Lai/賴信志, Sheng-Wei Pan/潘聖衛, Shih-Hsin Wu/吳世欣
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Nontuberculous mycobacterial lung disease (NTM-LD) has become an important clinical concern, because its incidence has increased, and high mortality as well as low treatment successful rate. However, the clinical relevance of NTM in sputum is only 33~42%, therefore, NTM-LD development supposedly indicates host susceptibility but the nature remains unclear at present**.** In particular, the functions of patient's macrophage (like TLR2 expression) and the response of T cells (like release of interferon-gamma) are important for immune defense but has both been reportedly decreased in patients with NTM-LD. The pathogenesis is still uncertain at present.

We identified significant gut microbiota dysbiosis, especially *Prevotella species* deficiency, existed in NTM-LD and closely correlated with disease severity. In the meantime, compromised TLR2 activity in feces and sera in NTM-LD patients was also highlighted. In mice model, enteral administration of *P. species* enhanced TLR2 signaling, restored immune compromise and ameliorated NTM-LD in gut-dysbiosis mice. Direct delivery using capsular polysaccharides (CPS) from *P. species* which enhanced TLR2 activation also subsequently reverse the susceptibility of NTM-LD.

However, the reduction of NTM pulmonary infection by *Prevotella* species supplement cannot be totally blocked by TLR2 inhibitor or in a TLR2 knocked-out mice model. The immune cell changes under *P. species* supplement had been investigated and might include an increase in dendritic cells in the colon, and macrophages in peripheral blood and lung tissue. CLEC"X" expression on dendritic cells and macrophage in blood, and TLR2 in dendritic cells as well as TLR2 ligand in colon were also increased by enteral supplementation *Prevotella* species.

In conclusion, *P. species* enteral supplementation might reverse NTM-LD susceptibility not only through TLR2 pathway. The immune changes from gut to lung suggested dendritic cells in colon and monocyte/macrophage in blood and lung increased and might play the key role for *P. species* related NTM protection. In addition, TLR2 ligand increased in colon and then TLR2 together with CLEC"X" pathway might interplay in this gut-lung axis.

Title of Project: Investigating the Mechanism of the Loss of NLRP12 Expression in Affecting NET Mediated-kidney Damage Through IFN Signature in Lupus Nephritis Patients

Project No.: NHRI-EX113-11234SI
P.I. Name: Szu-Ting Chen/陳斯婷
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Type I IFN signature is a hallmark of both pediatric and adult patients with systemic lupus erythematosus (SLE). In SLE patients, the expression of type I IFN genes and the IFN signature were induced by nucleic acids derived from cell debris components. Apoptosis and NETosis are frequently observed in SLE patients, where frequent apoptosis results from autoantibody-mediated tissue damage. Additionally, autoantibodies against ribonuclear proteins (RNPs) activate neutrophils, causing NETosis and mitochondrial DNA release. These nucleic acids not only induce type I IFN production via cytosolic nucleic acid receptors but act as autoantigens, leading to the generation of autoantibodies, which are important pathogenic factors in SLE patients. Furthermore, these self-derived nucleic acids bind with their corresponding antibodies to form the nucleic acid-containing-immune complex (NAIC), activating immune cells through Fcγ receptors. Our study focuses on NAIC-induced type I IFN signature, NET formation, and pathological change of mitochondria in neutrophils. We used bulk RNA sequence to investigate the gene profiles in neutrophils derived from SLE patients, where data showed that a strong IFN signature was observed in SLE patients. We explore why those neutrophils exhibit IFN signatures by examining the factors that induce IFN production and IFN-inducible genes, such as *ISG15*. Here, we showed that the NAICs derived from SLE serum induce ISG15 expression in neutrophils, and this induction is reduced when specific items are removed by treating the serum with enzymatic methods. These data suggest that either nucleic acid or protein components integrated into these NAICs contribute to an IFN signature to a certain extent. These NAICs also induce NET formation by triggering GSDMD cleavage, and thus, the N-GSDMD subunit targets the plasm membrane, resulting in DNA expulsion. Moreover, the NAIC stimulation triggers the change of mitochondrial membrane potential/mitochondrial hypo-polarization in a GSDMD-dependent manner, providing a hint that NAICs may induce mitochondrial pore formation by triggering GSDMD cleavage, where N-GSDMD targets the mitochondrial outer membrane. Therefore, the GSDMD inhibitor disulfiram (DSF) rescues this mitochondrial hypo-polarization, preventing mitochondrial DAN (mtDNA) release into the cytosol to trigger the subsequent type I IFN production. In animal study, we examined neutrophil recruitment to the glomerulus in various lupus models, including the pristane-induced lupus-like mouse model, a lupus-prone mouse model, and a model involving pristane injection into lupus-prone mice. Kidney sections harvested from mice at different ages, corresponding to different stages of disease progression, were evaluated for the frequency of neutrophil recruitment in response to the pathological changes observed in lupus disease progression. Here we showed that lupus-prone mice (lpr) with pristane injection exhibited Ly6G neutrophil infiltration and IgG deposition in the glomerulus. To investigate the role of NETosis in contributing to disease progression, DSF was orally taken every two days for one month. When lupus mice receiving DSF treatment exhibited a reduced frequency of double negative T cells in the spleen, reduced expression levels of CD40, and the transition frequency between B cells and plasma cells. These findings are associated with the reduced levels of autoantibody and serum MPO-DNA in DSF-treated lupus mice. In the histopathology, DSF-treated lupus mice present few levels of NET signal in the glomerulus compared to control lupus mice, as evidenced by staining the CitH3 and MPO. We also noted that prevention of NET formation by treating mice with DSF is beneficial for reducing IgG deposition in the glomerulus. Further experiments and mechanisms shall be addressed to explain these interesting observations.

Title of Project: Molecular Studies of the Myocardial Ischemia-associated Type I Interferon Induction

Project No.: NHRI-EX113-11235SI

P.I. Name: Helene Minyi Liu/劉旻禕
Key Professional Personnel: Wei-Xuan Lu/呂韋萱, Helene Minyi Liu/劉旻禕
Affiliation/Institution: National Taiwan University

Entire Project Period: From 2023 to 2025 (Total: 3 years)

The Roles of Macrophages in the Myocardial Infarction-Induced Damages to Cardiomyocytes

Myocardial infarction (MI) is an extremely dangerous condition with blockage of oxygenated blood flow to the heart. Due to the deprivation of oxygen, the ischemic cardiomyocytes are damaged and even undergo cell death. Previous studies have shown that damaged cardiomyocytes in MI regions would release damaged-associated molecular patterns (DAMPs) to stimulate macrophages to produce type I interferons (IFN). To identify molecules that served as DAMPs released from ischemic cardiomyocytes, we established an *in vitro* model by incubating murine cardiomyocyte HL-1 cells in hypoxia chamber and collecting the conditioned medium. We surveyed the DAMP molecules in the hypoxia conditioned medium (HCM), and we characterized the potent DAMPs to induce type I IFN in macrophages as heat-resistant molecules less than 3 kDa. We then pre-treated RAW264.7 cells with the cGAS inhibitor RU521 or the STING inhibitor H-151 to block type I IFN induction by HCM. However, pretreatment of RU521 was not able to block IFNB mRNA expression in RAW264.7 cells cultured with HCM. Thus, we assessed the concentration of 2'3'-cGAMP in HCM by ELISA and found that HCM contained high enough 2'3'-cGAMP to drive IFNB1 mRNA expression in RAW264.7 cell. We further utilized hypoxia co-culture of HL-1 and RAW264.7 cells to mimic MI condition when both cell types were under the hypoxia stress. We then observed that HL-1 cells cultured with RAW264.7 cells had a higher IFNβ mRNA expression and more protein expression of pro-apoptotic markers when compared to those of HL-1 cells cultured alone, suggesting that macrophage co-culture amplified the damage signals of cardiomyocytes under hypoxia. We then used an IFNAR-neutralizing antibody to block type I IFN signaling in hypoxia co-culture experiments and found that HL-1 cells treated with anti-IFNAR antibody rescued the progression of cardiomyopathy and cell apoptosis. These results suggested that type I IFN produced by macrophages may be one of the key factors causing cardiomyocyte damage during MI. Under hypoxia conditions, HL-1 cells co-cultured with immortalized bone marrow-derived macrophages (iBMs) recapitulated the phenotype as those co-cultured with RAW264.7 cells, and however, iBMs derived from TNFa/TBK1 double-knockout mice did not amplified the damage signals in co-cultured HL-1 cells. Our *in vivo* model also confirmed that macrophage depletion could mitigate cardiomyopathy and apoptosis in MI progression. In conclusion, we model that hypoxic cardiomyocytes may release 2’3’-cGAMP as the DAMPs to activate macrophages, inducing the production of type I IFN, which in turn further impacts the damage to cardiomyocytes. Our report suggests that blocking Anti-IFNAR treatment, such as anifrolumab for moderate to severe systemic lupus erythematosus, might also be beneficial in treating acute MI.

Title of Project: Structure-Based Drug Design for CFTR Chloride Channel
Project No.: NHRI-EX113-11236SI
P.I. Name: Tzyh-Chang Hwang/黃自強
Key Professional Personnel: Tzyh-Chang Hwang/黃自強, Rou-Shen Lin/林柔伸
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Inhibition of CFTR by VX-445, a CFTR corrector.

Cystic fibrosis (CF) is a common lethal genetic disease among Caucasians. One in every three thousand newborns is affected by CF, which results from mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. These mutations impair CFTR mRNA synthesis, CFTR protein expression, function, stability, or a combination of these factors. While CF manifests various clinical presentations, respiratory diseases primarily cause morbidity and mortality in these patients. Before 2019, the average lifespan for CF patients was 40 years, but the introduction of Trikafta by Vertex Pharmaceuticals increased this to over 50 years Trikafta benefits CF patients by improving lung function and extending lifespan. Elexacaftor (VX-445), a component of Trikafta, acts as a CFTR potentiator that enhances CFTR's channel function and a CFTR corrector that increases the expression of the CFTR protein in the cell membrane, yet our experiments have revealed an unexpected effect of VX-445: VX-445 also acts as a CFTR inhibitor. Using patch clamp techniques, we showed that VX-445 indeed increases whole-cell CFTR currents activated via the cAMP pathway. This potentiation effect inversely correlates with the level of cAMP stimulation. Notably, VX-445 inhibits rather than enhances WT-CFTR currents by approximately 10% when CFTR is maximally activated by protein kinase A and ATP. Mutating amino acid residues in the VX-445 binding site for correction did not abolish its inhibitory function, indicating a separate binding site for its inhibitory effect. These findings highlight VX-445's complexities and hence warrant further exploration and investigation. Understanding drug-CFTR interactions will advance future drug design for the treatment of CF and CFTR-related diseases.

Title of Project: Characterize Novel Regulators of Heart Regeneration Revealed by Comparative Time-ordered Gene Coexpression Network (TO-GCN)

Project No.: NHRI-EX113-11237SI
P.I. Name: Shih-Lei (Ben) Lai/賴時磊
Key Professional Personnel: Wei-Han Lang/郎偉涵, Chia-Lin Huang 黃嘉琳
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Myocardial infarction (MI) in humans causes irreversible loss of cardiomyocytes (CM) and fibrosis, leading to heart failure and death. Zebrafish can regenerate their hearts while close-related teleost medaka cannot, providing a unique platform to study heart regeneration by comparative analyses. We adopted a novel bioinformatic approach to compare the transcriptomic responses across species and constructed the regeneration-associated Time-Ordered Gene Coexpression Network (TO-GCN). Based on the transcriptional factors and their co-express genes in the regeneration-associated TO-GCN, we performed Reactome Pathway Analysis and identified pathways associated with metabolic activation/switch, cell proliferation, and immune response transition from innate to adaptive immunity. Since cardiomyocyte (CM) regeneration relies on the dedifferentiation of pre-existing CMs via reactivation of cardiac progenitor genes (*hand2*, *gata4*, *nkx2.5*, and *tbx20*) before cell cycle re-entry in both zebrafish and mouse, we characterized potential upstream regulators of cardiac progenitor genes in the regeneration-associated TO-GCN. We validated that candidate genes, including *nfic*, *sox3*, *srebf1*, and *tal1,* re-activated in FACS-sorted CMs from injured vs. untouched zebrafish hearts, and their expression colocalized with dedifferentiated CMs marker embCMHC. To test their function in heart regeneration, we generated *nfic* and *sox3* knockout zebrafish and found decreased CM dedifferentiation and proliferation, leading to unresolved scars in mutants compared to WT siblings after cardiac injury. To test their function in mammals, we found that shRNA knockdown of *Nfic* and *Srebf1* impaired the proliferation of primary CM isolated from P1 neonatal mice. Altogether, these results revealed key processes of heart regeneration and further identified evolutionarily conserved regulators essential for CM dedifferentiation and proliferation during heart regeneration. We are now preparing tools for gain-of-function experiments to test whether ectopic expression of these candidate genes can promote CM dedifferentiation and heart regeneration in non-regenerative medaka and mice as potential therapeutics.

Title of Project: Engineering of Therapeutic Cystine-knot Dimeric Proteins into Long-acting Single-chain Molecules

Project No.: NHRI-EX113-11238SI
P.I. Name: Ching-Wei Luo/羅清維
Key Professional Personnel: Ying-Wen Wang/王盈文, Chi-Ying Chen/陳祈瀅, Ching-Wei Luo/羅清維
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Cystine-knot proteins comprise many hormones and cytokines involved in metabolic diseases, aging and cancers of humans. Although being spotlighted as therapeutic proteins, challenges such as short physiological half-life and low efficiency in dimerization need to be overcome when engineering cystine-knot proteins to become protein drugs. We here proposed a new strategy that uses the C-terminal peptide (CTP), which is naturally derived from human chorionic gonadotropin and is capable of extending protein's half-life, to link two cystine-knot protein monomers. Vascular endothelial growth factor A-165 ($VEGF_{165}$), known to be critical for wound healing, was first selected as a target to demonstrate the feasibility of this design. Intriguingly, we demonstrated that single-chain $VEGF_{165}$ (V165-CTP-V165) is not only bioactive but also easy to be secreted. Pharmaceutical kinetic assay indicated that single-chain $VEGF_{165}$ induced downstream NFAT reporter activity as strong as its natural form, suggesting that they exhibit similar affinity and efficacy to VEGFR2. Also, using HUVEC and HMEC1 cells, bio-functional tests indicated that single-chain $VEGF_{165}$ induces downstream phosphorylation of AKT and ERKs, tube formation, and cell migration as strong as the commercial VEGF. We also preliminary found that single-chain $VEGF_{165}$ promotes the wound healing much better the commercial VEGF under single administration. Taken together, our proposed CTP linker provides a promising strategy to produce long-acting single-chain $VEGF_{165}$. More kinetic assays will be performed for confirmation.

Title of Project: Cardiac Ageing: Development of Novel Therapeutics for Age-related Heart Failure Using Cisd2 as a Molecular Target
Project No.: NHRI-EX113-11239SI
P.I. Name: Chi-Hsiao Yeh/葉集孝
NHRI Researcher: Jinq-Chyi Lee/李靜琪研究員
Key Professional Personnel: Ting-Fen Tsai/蔡亭芬, Jinq-Chyi Lee/李靜琪
Affiliation/Institution: Chang Gung Medical Foundation
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Cardiovascular disease holds the top position as the leading cause of death globally. With an aging global population, the incidence and mortality rates of cardiovascular disease and heart dysfunction are particularly high among seniors. Age-related cardiac dysfunction is compounded by multiple age-associated health issues, making it the primary cause of mortality in the elderly. This underscores the urgent need for effective treatments for age-related heart diseases.

Ischemic heart disease remains a leading cause of mortality worldwide. This study tests the hypothesis that enhancing Cisd2 activators can protect the heart from acute myocardial ischemia (AMI) injury by modulating mitochondrial bioenergetics and the protein translation initiation pathway. We examined mortality and heart failure following AMI by conducting loss- and gain-of-function experiments in cardiac-specific Cisd2-deficient and whole-body Cisd2 transgenic mice, with wild-type mice serving as controls. An AMI mouse model was constructed, and transcriptomes were profiled at the early stages of AMI progression. Cardiac function was evaluated using echocardiography. Cisd2 deficiency significantly exacerbated AMI mortality and cardiac dysfunction in mice. Conversely, consistent overexpression of Cisd2 prevented AMI-induced mortality and heart failure. Mechanistically, eIF2 was identified as a major downstream target of Cisd2 in the heart. Loss- and gain-of-function experiments demonstrated that eIF2 plays a key role in the Cisd2-mediated effects on calcium homeostasis and reactive oxygen species flux during AMI injury. Furthermore, hesperetin, as a proof of concept, along with CD0001 and CI0058 as Cisd2 activators, mimicked the effects of Cisd2 overexpression, ameliorating AMI injury and cardiac dysfunction. Persistent Cisd2 expression downregulates eIF2 phosphorylation, thereby controlling calcium homeostasis and endoplasmic reticulum stress translation initiation, ultimately improving cardiac function in a murine model of AMI. These findings provide new insights into the mechanisms underlying ischemic heart disease and suggest potential therapeutic targets for its treatment.

Title of Project: Molecular Mechanism of Focal Cortical Dysplasia Using Novel Genetic Screening Paradigm and Single-Cell Gene Expression Profiling
Project No.: NHRI-EX113-10904NI
P.I. Name: Jin-Wu Tsai/蔡金吾
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2020 to 2024 (Total: 5 years)

Haw-Yuan Cheng/鄭皓元 [1], Chen Liu/劉臻 [1], Chiao-Wen Nian/粘巧玟 [1], Hui-Chin Huang/黃惠勤 [1], Hong-Jun Zhao/趙紘均 [1], Fang-Shin Nian/粘芳馨 [1,2], Chien Chen/陳倩 [1,3], Chien-Yi Tung/董建億 [1], Wei-Yi Chen/陳威儀 [1], Yo-Tsen Liu/劉祐岑 [1,3], Pei-Shan Hou/侯珮珊 [1], and Jin-Wu Tsai/蔡金吾 [1]

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Focal cortical dysplasia (FCD) is a type of brain developmental disorder that features focal cortical malformations with altered cortical lamination. Using whole exon sequencing (WES), we identified a novel mutation in a novel forkhead box (FOX) transcription factor from a family with FCD and epilepsy. To explore the function of the FOX transcription factor during cortical development, we used in utero electroporation to knockdown the Fox gene in neural progenitor cells in mouse embryos. We found that Fox knockdown caused neural differentiation abnormality and cortical layering disorganization. After knockdown of Fox, cells change their cell fate from deep-layer neurons to up-layer neurons. Interestingly, cells supposed to become up-layer neurons retain their cell fate. Besides, Fox knockdown also maintained more cells in the cell cycle. Therefore, FOX deficiency affected cell proliferation, cell cycle regulation, and differentiation of mouse neural stem cells. By CHIP sequencing, we identified 6244 potential FOX binding targets, from which we found that the most enriched term of biological process was "cell cycle" with Gene Ontology analysis. To screen out the downstream targets of FOX transcription factor, we used RT-qPCR to check the regulation effect on those genes listed in the cortical development GO terms. Pten and Tsc1 were the potential candidates for further investigation. Rescue experiment was conducted by overexpressing Pten and Tsc1 in Fox knockdown cells. We found that Pten overexpression partially reversed the migration delay phenotype observed in FOX deficient cells. On the contrary, Tsc1 overexpression did not show rescue effects. This finding indicates that Pten, the negative regulator of AKT signaling pathway, serves as a downstream target of the Fox transcription factor and participated in the pathogenesis of FCD. In this study, we showed that Fox transcription factor controls cell cycle exit and differentiation of neural progenitors, thus maintaining cortical lamination during brain development. Pten plays an important role to help Fox transcription factor control neurogenesis stage in the cerebral cortex. These exciting findings may explain the mechanism of how Fox mutations cause FCD.

Keywords: cortical development; FCD; FOX; transcription factor, ChIP-sequencing, cortical lamination

Title of Project: CRISPR-mediated Base Editing for the Inner Ear Disorders
Project No.: NHRI-EX113-11005NI
P.I. Name: Yen-Fu Cheng/鄭彥甫
Key Professional Personnel: Chun-Ying Huang/黃淳瑩, Yi-Shiou Tsai 蔡易修, Jiun-Ying Huang 黃俊穎, Yu-Chi Chuang 莊育綺
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2021 to 2024 (Total: 4 years)

Deafness is one of the most common inherited sensory diseases in developed countries. Limited therapy is available for the deaf. Cochlear implantation is the only treatment choice for patients with severe to profound hearing loss. The outcomes of cochlear implantation vary from person to person. Therefore, it is imperative to develop new strategies to treat severe to profound hearing loss patients, especially those caused by genetic defects.

This proposal aims to locally deliver base editors [cytosine base editors (CBEs) and adenine base editors (ABEs)], which are carried by the AAV into the inner ear to treat hereditary hearing loss. Firstly, we evaluated whether base editors modified the nucleotides of β-catenin phosphorylation site *in vitro*. The results from Sanger sequencing showed base editors installed the mutation to block β-catenin phosphorylation. Further, we use exon 3-skipping for disabling the 3' splicing site (acceptor site) of intron 2 in β-catenin. This would promote nuclear translocation to activate Wnt/β-catenin signaling pathway. Immunoblot showed more abundance of β-catenin indicated a successfully disruption of the proteasomal degradation of β-catenin due to phosphorylation site changes. Co-transfection of intein-conjugated Cas9 and sgRNAs in cells can successfully recombine both terminals of Cas9 protein, leading to efficiently editing of the target nucleotides and thus increase of β-catenin activity. Finally, we tested AAV packaging of ABE components in a split-intein system for *in vivo* delivery. Although our data shown that the base editors carried by AAVs were expressed in cochlear cells, the editing efficiency *in vivo* was not obvious. This may be related to the transfection efficiency of different AAV capsids to cochlear tissue.

In this study, base gene editor was performed on common hearing loss genes in Taiwan including GJB2 and Slc26a4 mutations. We use of ABEs to correct a common GJB2 variant has a significant effect, with an editing efficiency of ~40%, and very low off-target effects (<0.5%). We are currently testing the editing effect of the split-intein system with ABEs on the GJB2 common mutation. We also use CBEs for correction of the Slc26a4 mutation, and preliminary results suggest that additional gene editors need to be tested. We have established two hearing-impaired mouse models and are conducting follow-up functional tests such as hearing and balance. The significance of the proposal will be the proof of concept for the gene therapy to treatment of hereditary hearing impairment caused by single gene mutation.

Title of Project: The Role of Glutamate Homeostasis in Glia Cells of the Epilepsy Disease
Project No.: NHRI-EX113-11006NC
P.I. Name: Cheng-chia Lee/李政家
Key Professional Personnel: Yi-Hsuan Lee/李怡萱, Hsiang-Yu Yu/尤香玉, Chien-chen Chou/周建成, Chun-Fu Lin/林俊甫, Syu-Jyun, Peng/彭徐鈞
Affiliation/Institution: Taipei Veterans General Hospital
Entire Project Period: From 2021 to 2024 (Total: 4 years)

Background and study goal Glial glutamate transporter GLT1 plays a key role in the maintenance of extracellular glutamate homeostasis. Recent human genetic studies have suggested that de novo mutations in GLT1 (EAAT2) cause early-onset epilepsy with multiple seizure types. Consistent with these findings, global GLT1 null mice show lethal spontaneous seizures. The consequences of GLT1 dysfunction vary between different brain regions, suggesting that the role of GLT1 dysfunction in epilepsy may also vary with brain regions. In this study, we aim to investigate the correlation of astrocytic GLT1 expression with physiopathological phenomena, including neuronal morphology, and hyperexcitation/inflammation-related astrocytes and microglia activation in the epileptogenic zone via resected human specimen, which proved by MR images, PET, vEEG, or SEEG.

Materials and methods The human specimen was collected from 40 patients with drug-resistant epilepsy (DRE), who underwent epilepsy surgeries for lesion resection. These patients underwent comprehensive presurgical evaluation, including each patient's clinical and noninvasive study such as semiology, scalp EEG, neuropychiatric test, PET, MEG, and SPECT. We will examine the human specimen using immunostaining analysis for GLT1, neuron marker NeuN, dendritic marker MAP2, reactive astrocyte marker GFAP, and microglia marker Iba1 to analyze the relationship between GLT1 expression and neuronal damage and gliosis. TUNEL and Fluoro-Jade C stain will be performed to determine the cell apoptosis and neuronal degeneration, respectively. The brain imaging, PET, EEG, neuropsychiatric test, and intraoperative ECoG will be interpreted and cross-correlated to the stain result. The brain connectivity study will also be discussed.

Progression Until now, we have collect 45 patients including 31 MR(+) and 14 MR(-) cases. The proceed of study is ahead to the schedule. We have already established standard GLT-1 staining method and quantify the staining molecules (AI assisted). The preliminary result showed positive relationship between the neuro-astrocyte coupling and the size of hippocampus. In these 2 years, we completed the statistics of Hypothesis 1.1, 1.3, 2.2, 2.3, 3.1. The results show that the smaller hippocampus volumes were tend to have poor glutamate buffering and neuro-astrocyte coupling, which also related to longer seizure onset period, duration and frequency. In neuro-cognitive study, some dominant side cognitive function (e.g. language function or naming function) were tend to have poor neuro-astrocyte coupling. Finally the functional connectivity of MR (+) and MR(-) patients were different, and this section are preparing to correlate with the sprouting of neuron or the function of glutamate buffering. However, we still need complete the patient collection and perform comprehensive statistics to prove the micro- and macro- relationship.

Title of Project: The Causal Correlation between Psychiatric Disorders and the Establishment of Cortical Functional Areas

Project No.: NHRI-EX113-11007NC

P.I. Name: Pei-Shan Hou/侯珮珊

Key Professional Personnel: Pei-Shan Hou/侯珮珊, Chih-Chieh Yang/楊智傑, Jin-Wu Tsai/蔡金吾

Affiliation/Institution: National Yang Ming Chiao Tung University

Entire Project Period: From 2021 to 2024 (Total: 4 years)

Psychiatric disorders arise from deficiencies in accurate cell components and clear boundaries during cortical development. The six-layered neocortex is crucial for higher-order behaviors like cognition and voluntary movements. Brodmann identified 52 functional areas within the neocortex, each with specific cell compositions and clear boundaries essential for a precise connectome. Proper cytoarchitecture in each area is established through common but variable molecular machinery during cortical development and is believed to be vital for developing psychiatric disorders. In this study, we assessed the local structural pattern by calculating the structural pattern index (SPI). We found the changed SPI were enriched in certain functional Brodmann areas, such as the BA4 primary motor area, BA13 insula, BA11 inferior orbitofrontal area, and BA23, BA 24, and BA 31 cingulate cortex. Genome-wide association tests revealed the genetic variants (SNPs) responsible for the local structural changes, and the SNP-mapped genes showed area preference in both human- and mouse-developing neocortical cells. Finally, a loss-of-function analysis of the *Morf4l1, Reep3, Tmed3,* and *Nrbf2* genes using the CRISPR/Cas9 system demonstrated the different impacts of the identified genes on migration behavior and laminar cell fate. These results suggested the contribution of identified genes in establishing accurate cell components during cortical development, which is critical for avoiding abnormal connectomes. Finally, our data demonstrated a pipeline for identifying local structural changes, associated genetic causes, and ways to evaluate potential pathological molecular mechanisms underlying mental disorders.

Title of Project: Neural Adaptive Deep Brain Stimulation in Treating Freezing of Gait in Parkinson's Disease: Explore the Efficacy and Mechanism

Project No.: NHRI-EX113-11104NI

P.I. Name: Chiung Chu Chen/陳瓊珠

Key Professional Personnel: Chiung-Chu Chen/陳瓊珠, Yi-Chieh Chen/陳翊捷, Hau-Tieng Wu/吳浩榳, Chih-Hua Yeh/葉智華, Ming-Dou Ker/柯明道, Po-Hsun Tu/杜柏勳, Po-Lin Chen/陳柏霖, Tzu-Chi Liu/劉子齊, Yi-Hui Wu/吳怡慧

Affiliation/Institution: Chang Gung University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Freezing of gait (FOG) is an intractable motor symptom in Parkinson's disease (PD). The response of FOG to dopaminergic medicine and deep brain stimulation (DBS) is variable. As FOG was improved by DBS in some patients, this intervention might paradoxically worsen the gait in others. The pathophysiology of this symptom remains largely unknown and render this symptom the unsolved challenged in the treatment of PD. How the basal ganglia neuronal oscillations related to FOG is a mystery. Thanks to the DBS surgery, it's possible to access the deep brain structure and measure the neural signals in real time in free-moving patients. In our previous studies, the onset of FOG in PD was associated with the dynamic change in oscillatory frequency band (~18 Hz) in local field potentials (LFP) recorded in subthalamic nucleus (STN). These results supported the hypothesis that dynamic change of beta oscillations of STN may contribute to the freezing in PD during gait tasks that trigger FOG.

Adaptive DBS was proved to be superior to the conventional, continuous stimulation in the treatment of bradykinesia and rigidity in PD. Whether the aDBS regime works for FOG is still unknown. In this study, synchronized STN LFPs from the implanted DBS electrodes were recorded, processed. The beta activities were utilized as a trigger biomarker and the stimulation only delivered when the beta range activity exceeded a certain threshold. The gait kinematics were recorded in PD patients, off-medication during forward walking, turning under two conditions: Simple and with dual-tasks. The effect of conventional and aDBS on the improvement of gait performance and how the difference between the beta oscillations between hemispheres (STNS) of all individuals correlate with the gait performance will be presented.

Title of Project: A Study on the Role of Slitrk1 in the Pathogenesis of Tourette Syndrome
Project No.: NHRI-EX113-11114NI
P.I. Name: Lih-Chu Chiou/邱麗珠
Key Professional Personnel: Jung-Chieh Du/杜戎玨, Man-Hsin Chang/張蔓欣, Chen-Jiun Yeh/葉宸濬, Ming-Tatt Lee/李鳴達, Hsin-Jung Lee/李欣蓉
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Tourette syndrome (TS) is a common childhood onset neuropsychiatric disorder marked by motor and vocal tics, significantly affecting patients' quality of life. Despite its prevalence, TS treatments are considered orphan drugs by the FDA due to their limited efficacy and significant side effects. The cortico-thalamic-basal circuitry dysfunction is implicated in pathogenesis of TS, though the exact mechanisms remain unclear. A postmortem study indicated a decrease in striatal interneurons, including cholinergic interneurons (ChIs), in TS patients. ChIs, which form only 1-2% of striatal neurons, extend dense networks crucial for striatal functions. Our research aims to validate the hypothesis that the protein, Slitrk1, encoded by the TS risk gene Slit and Trk-like 1 (Slitrk1) is vital for maintaining adult striatal ChIs functions and plays a role in TS pathogenesis. Initial evidence shows that silencing the Slitrk1 gene in adult mice striatal tissues reduces functional ChIs, resulting in TS-like tic behaviors. Slitrk1 knockdown (*Slitrk1*-KD) mice were generated via intra-striatal microinjection of short interfering RNA (siRNA) targeting Slitrk1 mRNA, with controls receiving scrambled siRNA. Post-72-hour Slitrk1 siRNA microinjection, *Slitrk1*-KD mice exhibited more and longer tic-like behaviors than controls, categorized into complex and simple tics. Sensorimotor deficits, an endophenotype of TS, were assessed using adhesive removal and acoustic startle prepulse inhibition (PPI) tests. *Slitrk1*-KD mice took longer to remove sticky tape from their forepaws and showed significant PPI impairment, mimicking TS patients' deficits. Monitoring over time showed more stereotypic behaviors in *Slitrk1*-KD mice than controls at Day-3 and Day-7, but not Day-10 post-injection, suggesting the knockdown effect lasts at least 7 days. Western blot analysis confirmed lower Slitrk1 protein levels in the striatum at Day-3 and Day-7, returning to normal by Day-10. Further investigation through immunofluorescence staining showed no significant difference in total ChIs numbers between *Slitrk1*-KD and control mice on Day-3, Day-7, or Day-10. However, the percentage of Slitrk1-positive ChIs significantly decreased in Slitrk1-KD mice on Day-3 and Day-7 but not on Day-10, correlating with TS-like behaviors and decreased neuronal activity, as evidenced by reduced the percentage of phosphorylated S6 ribosomal protein (pS6RP) co-immunoreactivity in ChIs. Electrophysiological studies revealed differences between Slitrk1-positive and Slitrk1-negative ChIs, with Slitrk1-negative ChIs exhibiting higher membrane input resistance, smaller membrane capacitance, and different action potential properties. These findings suggest that Slitrk1 protein regulates the electrophysiological properties of striatal ChIs, and its absence disrupts downstream neuronal functions, leading to stereotypic tic-like behaviors. Microdialysis experiments comparing evoked and basal acetylcholine (ACh) levels in striatal microdialysates between *Slitrk1*-KD and control mice showed impaired evoked ACh release in *Slitrk1*-KD mice when exploring a novel environment, while basal ACh levels remained unchanged, underscoring the role of Slitrk1 in regulating ChIs function. These results highlight the critical role of Slitrk1 in maintaining striatal ChIs function and its involvement in TS pathogenesis. Reducing Slitrk1 protein in ChIs impairs their function, leading to TS-like behaviors in mice. This study provides a foundation for developing targeted TS therapies focusing on preserving or restoring Slitrk1 function in striatal ChIs.

Title of Project: The Role of TRPM2 in Inflammation-associated Neurocognitive Disorders
Project No.: NHRI-EX113-11115NC
P.I. Name: Chun-Hsiang Tan/譚俊祥
Key Professional Personnel: Chun-Hsiang Tan/譚俊祥, Rwei-Ling Yu/余睿羚
Affiliation/Institution: Kaohsiung Medical University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Background and aim:

We hypothesized that the transient receptor potential melastatin 2 (TRPM2) ion channel is critical in inflammation-associated neurocognitive disorders. Preliminary results from the first two years indicated that global TRPM2 deletion reduced spatial learning and memory impairments following lipopolysaccharide (LPS)-induced inflammation. This year, we aim to elucidate the mechanisms by which TRPM2 modulates these disorders, focusing on its role in different cell types, including cortical neurons and microglia.

Methods:

Conditional TRPM2 knockout models were developed to specifically delete TRPM2 in cortical neurons and microglia using floxed TRPM2 mice and Cre recombinase drivers. Tamoxifen was used for inducible knockout in microglia. Hematopoietic cell-specific TRPM2 knockout mice were also generated. Following LPS-induced inflammation, cognitive functions were assessed via the Barnes Maze, and motor functions were evaluated using rotarod and pole tests. Cre-driver lines were validated by crossing with tdTomato reporter mice, and TRPM2 expression was examined using immunohistochemistry.

Results:

No significant differences in Barnes Maze, rotarod, and pole test performance were observed between microglia-specific TRPM2 knockout mice and their littermates. Immunohistochemical analysis confirmed the absence of TRPM2 expression in microglia, explaining the lack of impact from its deletion. Preliminary behavioral data on cortical neuron-specific TRPM2 knockout mice were obtained, but further analysis is needed to determine significance.

Conclusion:

Global TRPM2 deletion protects against LPS-induced cognitive impairments. However, TRPM2 deletion in microglia does not affect neurocognitive functions due to the absence of TRPM2 expression in microglia, contrary to previous reports. These findings highlight the complexity of TRPM2's role in neuroinflammation and suggest its impact may be cell-type specific. We will continue to investigate the role of TRPM2 in cortical neurons and other cell types to elucidate its mechanisms and potential as a therapeutic target for inflammation-associated neurocognitive disorders within the remaining duration of our project.

Title of Project: Mechanisms and Intervention for Vascular Cognitive Impairment
Project No.: NHRI-EX113-11132HT
P.I. Name: Chaur-Jong Hu/胡朝榮
NHRI Researcher: Hung-Yi Chiou/邱弘毅
Key Professional Personnel: Yi-Chen Hsieh/謝宜蓁, Joen-Rong Sheu/許準榕, Chih-Hao Yang/楊志豪, Sung-Chun Tang/湯頌君
Affiliation/Institution: Taipei Medical University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Vascular dementia or vascular cognitive impairment (VCI) following Alzheimer disease (AD) is the second common cause of dementia. Coexisting of AD pathology, in terms of amyloid and tau accumulation and brain injury due to cerebral vascular diseases is common and increasing along the aging. The mechanisms of VCI are not totally clear and there is no effective treatment for VCI currently. This project including 4 Component projects, 1. Combined clinical and biomarkers to develop prediction model for post-stroke cognitive impairment, 2. Mechanisms and Intervention of Brain Glymphatic System in Vascular Cognitive Impairment, 3. Microglia mediated neuro-inflammation in cognitive impairment after ischemic brain injury. 4. Cellular Senescence and Senolytic Therapy in Vascular Cognitive Impairment. **Component project 1:** A total of 588 ischemic stroke patients had been enrolled. Among them, patients with post-stroke cognitive impairment (PSCI) exhibited higher rates of age, heart disease, and diabetes and lower body mass index (BMI). The blood-based biomarkers study revealed significantly elevated plasma levels of sCD137 and CSCL13 in PSCI patients. The senescence-associated secretory phenotype (SASP) index scores were different between groups. PSCI patients had a significantly higher percentage of elevated cognitive impairment-related polygenic risk scores (PRS). **Component project 2:** A MRI-based glymphatic system measurement method for VCI animal model, chronic bilateral common carotid artery occlusion (BCCAO), was established. After injection of Gd-contrast median into 4th ventricle, the contrast medium wash-in rate and wash-out rate in brain were reduced and contrast medium remains at 300 min after injection increased by BCCAO. It implicated glymphatic impairment in BCCAO animals. In human study, we found glymphatic function, diffusion-tensor-image analysis along the perivascular space (DTI-ALPS) index is correlated with cognitive functions among PSCI patients and it might be associated with hemodynamic parameters, measured in cerebral vessels. These results support hemodynamic changes induced by cerebrovascular diseases play a role in glymphatic dysfunctions. **Component project 3:** In acute ischemic stroke and chronic cerebral hypoperfusion (CCH) models, we identified key interactions between hyper-reactive microglia with glymphatic structures. Our findings highlighted the impairment of glymphatic clearance, decreased astrocytic endfeet coverage on blood vessels, and reactive microglial infiltration into the perivascular space post-stroke. In stroke models with rehabilitation therapy and microglia-specific transcriptomic analyses, we identified TRIM5 as a crucial mediator which exhibited the most prominent regulation, localizing to microglial cells interacting with glymphatic structures. Using TRIM5 knockout mice, we demonstrated that the absence of TRIM5 ameliorated stroke-induced motor, cognitive, and emotional impairments. **Component project 4:** In *in vitro* ischemia model, oxygen-glucose deprivation/reoxygenation (OGD/R) significantly increased senescence-associated beta-galactosidase (SA-ß-gal) staining in astrocytes and microglia, with minor effects on neurons. *In vivo* CCH model, bilateral carotid artery stenosis (BCAS) induced elevated p21 expression accompanied by increased double-positive p21/Iba1 and p21/NeuN cells. BV2 cell-conditioned media/exosomes induced senescence and upregulated SASP in naïve BV2 cells. The senolytic cocktail dasatinib and quercetin (D+Q) selectively eliminated senescent BV2 cells. These findings highlight the induction of cellular senescence in stroke and VCI models.

Title of Project: Novel Treatment for Ultra-resistant Schizophrenia: Dual Modulation of NMDA Receptor and Kynurenine Pathway

Project No.: NHRI-EX113-11133NI

P.I. Name: Hsien-Yuan Lane/藍先元

Affiliation/Institution: China Medical University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Background: At present, the efficacy of pharmacotherapy for schizophrenia remains limited. Furthermore, there has been no treatment for ultra-resistant schizophrenia. Therefore, new drug development is urgently needed. We are the first group to discover that D-amino acid oxidase (DAAO) inhibitor A drug can reduce positive and negative symptoms of ultra-resistant schizophrenia, but cannot improve cognitive function. On the other hand, patients with schizophrenia have higher blood levels of kynurenine (KYN), which is related to the patient's pro-inflammation, autoimmunity, insufficient NMDAR neurotransmission, and poor treatment response. In addition, B drug, with anti-inflammatory and KYN-reducing effects, is also used for adjuvant treatment of schizophrenia, though the efficacy is also limited. Therefore, addition of B drug to A drug would enhance the treatment for ultra resistant schizophrenia.

Methodology: This 3-year project will enroll 40 or more patients with ultra-resistant schizophrenia. They keep their original treatment and are double-blindly randomized into two treatment groups for 12 weeks: (1) A drug (1 g/day) plus placebo, or (2) A drug (1 g/day) plus B drug (1-4 g/day). We measure clinical performances at weeks 0, 2, 4, 6, 9, and 12 using Positive and Negative Syndrome Scale (PANSS), Scale for Assessment of Negative Symptoms (SANS), Clinical Global Impression (CGI), Hamilton Depression Rating Scale (HDRS), Quality of Life scale, Global Assessment of Function (GAF), and scales of side effects. At week 0 and endpoint, we assess 7 cognitive domains recommended by National Institute of Mental Health, USA (processing speed, sustained attention, working memory, verbal learning/memory, visual learning/memory, reasoning and problem solving, and social cognition) and assay NMDAR- and KYN pathway-related biomarkers.

Results: During the first 2.5 years, we have enrolled 35 patients. This ongoing study has not yet been decoded. The patients improved in all clinical measures. The mean PANSS total score reduced from 78.0 ± 9.2 at baseline to 71.5 ± 8.4 at endpoint. The mean PANSS positive subscale scores reduced from 16.3 ± 4.1 to 15.2 ± 3.9. The PANSS general psychopathology reduced from 38.3 ± 5.2 to 34.4 ± 5.0. SANS reduced from 48.0 ± 10.7 to 42.0 ± 8.7. CGI reduced from 4.4 ± 0.5 to 4.1 ± 0.5. HDRS reduced from 10.7 ± 5.5 to 9.0 ± 5.7. Quality of Life improved from 42.1 ± 15.9 to 46.5 ± 16.0; and GAF improved from 46.0 ± 7.7 to 51.4 ± 7.9. Seven cognitive domains were also assessed. However, the scores need to be converted into T-scores after we complete the subject enrollment. Therefore, score changes have not yet been calculated. To date, the treatment yielded no evident side effects. In laboratory, we assayed WBC mRNA levels of NMDA-related genes, SRR, PSAT, GCAT, GAD1, CSRP1, AMT, etc. Moreover, KYN, kynurenic acid, indoleamine 2,3-dioxygenase 1, tryptophan dioxygenase, kynurenine aminotransferase II, DAAO, and G72 (DAAO activator) proteins were determined by ELISA kits. Because the data has not yet been decoded, the results of the analysis and the between-group comparison will be conducted after the whole study is completed. To date, 18 articles have been published.

Conclusions: The preliminary result suggests that combination of A and B drugs could reduce symptoms and improve life quality in patients with ultra-resistant schizophrenia. Final clinical and laboratory results will be revealed after we complete and decode the study. We expect that the results will help the development of novel treatments and precision medicine of ultra-resistant schizophrenia.

Title of Project: Neural Control of Mitophagy in Aging and Stress Resistance
Project No.: NHRI-EX113-11134NI
P.I. Name: Chun-Liang Pan/潘俊良
Key Professional Personnel: Y-Cheng Chang 張郁承, En-Ni Chang/張恩妮, Chin-Mei Lee 李金美
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Intercellular regulation of mitochondrial morphology and function is important for physiological homeostasis at the organismal level. Our previous study found that inhibition of mitochondrial fusion in *C. elegans* neurons, via depletion of *fzo-1*/Mitofusin, triggers systemic mitochondrial fragmentation in distal non-neural tissues through serotonin and tyramine. This non-autonomous control of mitochondrial connectivity engages autophagy/mitophagy genes and enhances pathogen immunity. Here we investigated the molecular mechanisms by which that tyramine and serotonin promote non-autonomous mitochondrial fragmentation in the intestine. Tyramine targets the TYRA-3 G-protein coupled receptor in intestinal cells, and it also acts via LGC-55, a tyramine-gated chloride channel, in the neurons. On the other hand, serotonin mainly propagates within the neural network via the MOD-1 ligand-gated chloride channel in the GABAergic neurons to promote intestinal mitochondrial fragmentation. Tyramine-TYRA-3 signaling likely engages EGL-30, the sole *C. elegans* G alpha q. Both tyraminergic and serotonergic signaling promotes nuclear translocation of HLH-30/TFEB, a transcription factor required for lysosome and autophagy function, in the intestine. We previously found that autophagy genes are upregulated by neuronal *fzo-1* depletion. Extending this observation, we showed here that *sqst-1/p62*, *atg-9*, *epg-9* and *dct-1/BNIP3* are required for non-autonomous mitochondrial fragmentation triggered by neuronal *fzo-1* depletion. Importantly, neuronal stress via *fzo-1* knockout enhances resistance against pathogen stress of *Pseudomonas* infection, and this improved pathogen immunity requires tyraminergic and serotonergic signaling, as well as HLH-30. Our findings uncover a tyramine/serotonin-TFEB-autophagy signaling network for brain-gut communication that regulates mitochondrial connectivity to enhance stress resistance and pathogen immunity.

Title of Project: Interrogation of the Function of Inter-hemispheric Hippocampal Inhibition in Contextual Memories

Project No.: NHRI-EX113-11135NI

P.I. Name: Cheng-Chang Lien/連正章

Key Professional Personnel: Cheng-Chang Lien/連正章

Affiliation/Institution: National Yang Ming Chiao Tung University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

The dentate gyrus (DG) receives substantial input from the homologous brain area of the contralateral hemisphere. This input is by and large excitatory. Viral-tracing experiments provided anatomical evidence for the existence of GABAergic connectivity between the two DGs, but the function of these projections has remained elusive. Combining electrophysiological and optogenetic approaches, we demonstrate that somatostatin-expressing contralateral DG (SOM+ cDG)-projecting neurons preferentially engage dendrite-targeting interneurons over principal neurons. Single-unit recordings from freely moving mice reveal that optogenetic stimulation of SOM+ cDG projections modulates the activity of GABAergic neurons and principal neurons over multiple timescales. Importantly, we demonstrate that optogenetic silencing of SOM+ cDG projections during spatial memory encoding, but not during memory retrieval, results in compromised DG-dependent memory. Moreover, optogenetic stimulation of SOM+ cDG projections is sufficient to disrupt contextual memory recall. Collectively, our findings reveal that SOM+ long-range projections mediate inter-DG inhibition and contribute to learning and memory.

Title of Project: Exploring The Role of Gut Microbiota Metabolite Short Chain Fatty Acids in the Pathophysiology and Treatment Strategy of Parkinson'S Disease

Project No.: NHRI-EX113-11136NI

P.I. Name: Chin Hsien Lin/林靜嫻

Key Professional Personnel: Ming-Shiang Wu/吳明賢, Hsin-Chih Lai/賴信志, Hsiao-Li Chuang/莊曉莉

Affiliation/Institution: National Taiwan University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Parkinson disease (PD) is a common neurodegenerative progressive motor dysfunction disorder in aging society. The pathological hallmarks of PD, neuronal a-synuclein accumulations as Lewy body, may start from the gut enteric nervous system and then spreading to the central dopaminergic neurons through gut-brain axis. Gastrointestinal dysfunction, especially constipation, precedes motor symptoms by decades and is regarded as prodrome symptoms of PD. Although altered gut microbiota between patients with PD and healthy controls is well recognized, how changes of gut microbiota integrated with gut metabolites impacting neurodegeneration in PD remain unknown. Gut metabolites produced by gut bacterial fermentation of dietary components are speculated to be pivotal in gut–brain cross talks. Among these gut metabolites, short chain fatty acids (SCFAs) has been shown to associate risk and severity of patients with PD. Plasma levels of SCFAs were recently shown to promote neuropathology in a PD rodent model. However, the results of fecal levels of SCFA in human PD patients were reduced. These controversy findings prompted us to concomitantly examine the plasma and fecal levels of SCFAs in a cohort of PD patients and age/sex/diet habits-matched controls. We observed an increased plasma but reduced fecal levels of SCFAs in PD patients compared to controls. Notably, the increased plasma/fecal ratios of SCFAs correlated with clinical several and the abundances of SCFA-producing microbiota, including *Clostridaceae bacterium*, *Clostridium butyricum* and *Rosburia intestinalis*. We therefore hypothesize that these gut metabolites may leak from gut epithelium into the systemic circulation and then cross the blood-brain barrier to promote neuropathology of PD through activating free fatty acid receptors. Using both *in vitro* and *in vivo* models of PD, we have demonstrated that high dose of SCFAs promoted neurodegeneration in both enteric and central dopaminergic neurons leading to aggravated motor dysfunction through activating specific free fatty acid receptors. High dose of SCFAs accelerates the a-synuclein production in both transcriptional and translational level, leading to increased aggregated a-synuclein fibrils within cells. Our results will elucidate the effects of gut metabolites, focusing on SCFAs, in the neurodegeneration process of PD.

Title of Project: The Association among Monocyte/Macrophage of the Innate Immunity, Antipsychotics Exposure, and Vascular Atherosclerosis In Schizophrenic Disorder

Project No.: NHRI-EX113-11201NI
P.I. Name: Shang-Ying Tsai/蔡尚穎
Key Professional Personnel: Cheng-ying Hsieh/ 謝政穎, Pao-Huan Chen/陳抱寰
Affiliation/Institution: Taipei Medical University
Entire Project Period: From 2023 to 2027 (Total: 5 years)

Objectives: Atherosclerosis is the vital pathological basis for most cardiovascular diseases (CVD), which is principal cause of natural mortality and health threat for patients with schizophrenia (SCZ). The evidence for monocyte/macrophage activation along with antipsychotics-treatment linking with atherosclerosis in SCZ are lacking. The aim of this case-control study is to explore the association among atherosclerosis, innate immunity inflammatory markers, parameters of oxidative stress, and effects of the second generation antipsychotics (SGAs) treatment.

Method: The physically healthy outpatients aged 20-60 years with schizophrenic disorder (DSM-5) and age-matched bipolar I disorder (BPD) along with mentally normal adults as control groups were recruited. All individuals with substance abuse, a body mass index (BMI) over 30 kg/m², or pregnancy were excluded. We measured carotid intima-media thickness (CIMT) via M-mode ultrasound and plasma levels of macrophage/monocyte specific marker, chemokines, soluble cytokine receptors, and parameters of oxidative stress. Patient's clinical data were obtained by reviewing available medical charts and directly interviewing.

Results: A total of 106 SCZ patients with mean 44.6 years old completed all the laboratory and carotid ultrasound examinations. Patients with SCZ and BPD exhibited significantly greater CIMT and elevated uric acid levels compared to normal controls. The mean level of hs-C reactive protein in SCZ group was significantly higher than that of BPD and NC groups. Within the SCZ group, CIMT was significantly correlated with soluble form of CD40 ligands (sCD40L) ($\gamma = 0.38$), interleulin-8 ($\gamma = 0.39$), oxidized low-density lipoprotein (ox-LDL) ($\gamma = 0.54$), and superoxide dismutase (SOD) ($\gamma = -0.40$). Notably, there was no significant relationship between CIMT and any SGA-medication variables such as mean daily dosage, drug exposure ratio, and years of lifetime treatment. After adjusting for age and BMI, multiple stepwise regression found that higher levels of ox-LDL were significantly associated with increased CIMT, explaining 28.4% of the variance ($\beta = 0.41$, $t = 3.09$, $p = 0.004$).

Conclusions: Patients with SCZ display accelerated atherosclerosis and were accompany with activation of inflammatory response system. The oxidative damage due to ox-LDL on vascular endothelium may play a rather important role than SGAs in acceleration of arterial atherosclerosis in SCZ. However, the association between SGAs and oxidative stress along with SCZ-specific inflammatory activation need further investigation.

Title of Project: Mechanistic Link from Amyloidosis to Tauopathy In Alzheimer's Disease: Role of Glutamate Transporter

Project No.: NHRI-EX113-11207NI
P.I. Name: Yu-Min Kuo/郭余民
Key Professional Personnel: Tzu-Feng Wang/王姿丰
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2023 to 2026 (Total: 4 years)

The “A/T/N” criteria define Alzheimer's disease (AD) based on its underlying pathology, reflecting a chronological sequence from amyloid plaque deposition to neurofibrillary tangle formation, leading to neurodegeneration and cognitive decline. By conducting analyses of glia-specific expression residuals in the ROSMAP and MAYO cohorts, we pinpointed EAAT2 as master regulators of AD-associated genes. EAAT2 and EAAT1 (collectively, EAAT1/2), are predominantly expressed in astrocytes and decreased EAAT1/2 levels contribute to excitotoxicity. Aβ-induced excitotoxicity activates p25/Cdk5, leading to tau phosphorylation and neurodegeneration. To delineate the roles of EAAT1/2 in regulating excitotoxicity, Cdk5 activity and tau phosphorylation, we injected AAVs expressing shRNA against EAAT1/2 into the dorsal hippocampus (dHPC) of 3xTg-AD mice at the age of 13 months, while mice received AAVs expressing shLacZ served as the control group. Our results showed that reduction in EAAT1/2 expression impaired dHPC-related spatial learning and memory. Notably, the reduction of EAAT1/2 triggered local Cdk5 activation, indicated by an increase in the expression ratio of p25 to p35, and exacerbated tauopathy without affecting amyloidosis. To test the effect of neuronal excitotoxicity on tauopathy, we administered kainic acid (KA, 20 mg/kg, i.p., daily for three days), an agonist of the glutamatergic kainate receptor, to 16-month-old 3xTg-AD mice. We found that this KA treatment induced neuronal excitotoxicity and Cdk5 activation by increasing the expression ratio of p25 to p35 in the dHPC of these mice. Moreover, KA worsened tauopathy but did not influence amyloidosis in the dHPC of 3xTg-AD mice, implying that glutamate-mediated excitotoxicity plays a role in the development of tauopathy. To further examine the involvement of EAAT1/2 in the progression of AD pathologies, we found an FDA-approved drug (riluzole, RLZ) recognized for its ability to increase the expressions of EAAT1/2. RLZ (4 mg/kg, i.p.) was daily given to 15-month-old 3xTg-AD mice over a period of 28 days. The results showed that RLZ effectively elevated the levels of EAAT1/2, suppressed Cdk5 activity, and mitigated tauopathy and amyloidosis in the dHPC. These findings suggest that increased levels of EAAT1/2 can inhibit excitotoxicity and hold AD-related pathologies at A^+/T^- stage. Moreover, RLZ could be a promising therapeutic agent for the treatment of AD. In summary, our findings support our hypothesis that excitotoxicity caused by EAAT1/2-related defects in glutamate clearance represents a pathogenic link between “A” and “T”. Our findings also offer potential drug choices that may arrest AD pathology in the A^+/T^- stage. As AD pathology PET is readily for clinical usage, the strategy of holding AD pathology in the A^+/T^- stage is not only feasible, but also has important clinical significance.

Title of Project: Investigating ACD Regulators (Insc/Lgn/Par3) in Mediating Microtubule Stability for PNS Degeneration

Project No.: NHRI-EX113-11228NI

P.I. Name: Chih-Chiang Chan/詹智強

Key Professional Personnel: Jui-Yu Yeh, Hua-Chuan Chao, Cheng-Li Hong, Yu-Chien Hung, Fei-Yang Tzou, Cheng-Tsung Hsiao, Jeng-Lin Li, Wen-Jie Chen, Cheng-Ta Chou, Yu-Shuen Tsai, Yi-Chu Liao, Yu-Chun Lin, Sue-wei Lin, Shu-Yi Huang, Marina Kennerson, Yi-Chung Lee

Affiliation/Institution: National Taiwan University

Entire Project Period: From 2023 to 2025 (Total: 3 years)

PAR3/INSC/LGN form an evolutionarily conserved complex required for asymmetric cell division in the developing brain, but its post-developmental function and disease relevance in the peripheral nervous system (PNS) remains unknown. We mapped a new locus for axonal Charcot–Marie-Tooth disease (CMT2) and identified a missense mutation c.209 T > G (p.Met70Arg) in the *INSC* gene. Modeling the $INSC^{M70R}$ variant in *Drosophila*, we showed that it caused proprioceptive defects in adult flies, leading to gait defects resembling those in CMT2 patients. Cellularly, PAR3/INSC/LGN dysfunction caused tubulin aggregation and necrotic neurodegeneration, with microtubule-stabilizing agents rescuing both morphological and functional defects of the $INSC^{M70R}$ mutation in the PNS. Our findings underscore the critical role of the PAR3/INSC/LGN machinery in the adult PNS and highlight a potential therapeutic target for *INSC*-associated CMT2.

Title of Project: Transcranial Focused Ultrasound(Tfus) Neuromodulation on Epilepsy: from Epileptogenic Network Investigation to Intervention for Drug-Resistant Epilepsy

Project No.: NHRI-EX113-11229NI

P.I. Name: Hsiang-Yu Yu/尤香玉

Key Professional Personnel: Hsiang-Yu Yu/尤香玉, Chien-Chen Chou/周建成, Yen-Cheng Shih/施彦丞, Cheng-chia Lee/李政家, Wen-Jui Kuo/郭文瑞

Affiliation/Institution: Taipei Veterans General Hospital

Entire Project Period: From 2023 to 2025 (Total: 3 years)

Epilepsy is a disease characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition. Drug-resistant epilepsy (DRE) brings negative impact on patients' quality of life and social function. Resective surgery is a good solution for surgery remediable cases. However, resective surgery is not suitable for every case of DRE. Neuromodulation is an alternative way to reduce seizure burden for patients with DRE. In addition to electric stimulation (vagus nerve stimulation, deep brain stimulation, transcranial direct current stimulation) and magnetic stimulation (transcranial magnetic stimulation), transcranial focused ultrasound (tFUS) is an emerging brain stimulation which has a novel mechanisms (mechanical and thermal) and less invasiveness over present brain stimulation methods. Our group has finished a phase one study of tFUS neuromodulation for patients with DRE with concomitant intracranial EEG (iEEG) recording. The iEEG showed power change after tFUS treatment. It has potential in the treatment of DRE. We would like to extend our research to investigate the epileptogenic network modulated by tFUS, via using corticocortical evoked potential (CCEP) and to evaluate the efficacy of neuromodulation via functional neuroimaging for epilepsy developed by our group. There are two specific aims in this study. In Aim 1, adult patients who have finished their stereo-EEG recording with a conclusion of seizure onset zone (SOZ) will be recruited. We will perform single-pulse CCEP to DRE patients before and after a 5- minute tFUS stimulation. The second aim was to evaluate the treatment effect of tFUS by using a new EEG-fMRI method developed and validated in our group, the fast fMRI acquisition (ten brain volumes per second) in concurrent EEG-fMRI recording to sensitively and accurately delineate the irritative zone by reducing EEG artifacts. In our phase II trial, a single blind, randomized crossover study enrolled 12 patients to evaluate the safety and efficacy of tFUS neuromodulating treatment for patients with drug resistant epilepsy. We collected the data of EEG-fMRI before tFUS treatment and hours after tFUS treatment. Seven cases (3 male, 4 female, age 21-56 years) completed the EEG-fMRI examination. One of the cases who showed response to tFUS whose seizure was from the left temporal lobe. The default mode connectivity seemed to show reduced connectivity with brain regions in the temporal lobe after tFUS treatment. A reduction of salience network was also noted. Further analysis was ongoing and will be shown in the meeting.

Title of Project: Investigating the Causal Mechanism of Developmental Anomaly of the Corpus Callosum in Neuropsychiatric Disorders

Project No.: NHRI-EX113-11230NI
P.I. Name: Guey-Shin Wang/王桂馨
Key Professional Personnel: Lee-Hsin Wang/王李馨
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2023 to 2025 (Total: 3 years)

The corpus callosum connects the left and right hemispheres and functions in integration, transfer and processing of the sensory, motor and cognitive information from both hemispheres. Defects in the corpus callosum is the most common pathological feature among neurological diseases including neurodegenerative, neurodevelopmental and neuropsychiatric diseases. Corpus callosum atrophy is strongly associated with cognitive decline in neurodegenerative diseases, whereas partial agenesis of the corpus callosum is commonly seen in neurodevelopmental and psychiatric disorders, suggesting a possibility for the callosal projection neuron with an intrinsic vulnerable characteristic. However, the cause of predisposition to malformation or breakdown of the corpus callosum in neurological diseases remains undetermined. The cognitive impairments of myotonic dystrophy type 1 (DM1) include mental retardation, autism spectrum disorder (ASD), depression, attention deficit hyperactivity disorders (ADHD), and neurodegeneration. Hypoplasia and atrophy of the corpus callosum are the major feature of DM1 brain. The genetic basis of DM1 is caused by an expansion of CTG repeats in the 3' untranslated region (UTR) of the Dystrophia Myotonica Protein Kinase (*DMPK*) gene. *DMPK* mRNA containing expanded CUG repeats accumulates in nuclear foci and disrupts functions of at least two families of RNA binding proteins: muscleblind like (MBNL) and CUGBP Elav-like family member (CELF) proteins. To understand the impact of expanded CUG (CUG^{exp}) RNA on the cortical development, we established a mouse model, EpA960/$Emx1^{IRES\mathit{cre}}$, for expression of CUG^{exp} RNA in neural progenitors of the dorsal telencephalon. Embryonic expression of CUG^{exp} RNA induced cell death in neural progenitors during neurogenesis, leading to a reduction in the generation of callosal projection neurons (CPNs). This resulted in hypoplasia of the corpus callosum and decreased cortical thickness, which were associated with learning and memory deficits. Transcriptome analysis of EpA960/$Emx1^{IRES\mathit{cre}}$ embryonic brain revealed a down-regulation of genes involved in cell proliferation and differentiation. Examination of the axonal trajectory of CPNs in EpA960/$Emx1^{IRES\mathit{cre}}$ brain by DiI labeling and L1 immunostaining revealed a disorganized axonal trajectory and a reduction in midline crossing of CPNs to the contralateral cortical region. To determine whether there are intrinsic characteristics that dictate the susceptibility of CPN progenitors to cell death, we aim to identify the population of progenitors that are susceptible to CUG^{exp} RNA-induced cell death using single-cell RNA sequencing (scRNA-seq) approach. First, we are establishing the transcriptome profiles of developing mouse cortex using publicly available scRNA-seq data, and will identify the lineage of CPNs during cortical development from the analyses. These profiles will serve as a reference for later comparison. Dissociated cells collected form the dorsal telencephalon of EpA960/$Emx1^{IRES\mathit{cre}}$ brain at different time points will be used for scRNA-seq. By comparison of the results with the established referenced transcriptome profiles will help identify the population of susceptible progenitors.

Title of Project: Modulation of Emotional Valence and Emotion Processing in Human Empathy
Project No.: NHRI-EX113-11231NI
P.I. Name: Ming-Tsung Tseng/曾明宗
Key Professional Personnel: Min-Min Lin/林敏敏, Zhilin Su/蘇致霖, Ming-Tsung Tseng/曾明宗
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Modulation of Emotional Valence and Emotion Processing in Human Empathy: the Role of Social Comparison in Empathic Responses

Empathy, the capacity to share the emotions of others, plays an important role in human social interaction, and impaired empathic responses are present in many neuropsychiatric disorders. Preliminary research shows that social comparisons, a process that we compare a target (i.e., ourselves) to a certain standard (i.e., another person), may influence human empathic responses, but the underlying neural mechanisms remain unclear. Evidence indicates that empathy for others' pain experiences activates the anterior insular cortex (aIC), a key brain region implicated in processing first-hand painful experiences. Interestingly, another line of evidence shows that aIC was also involved in upward comparisons (that is, one's own rank on a given comparison dimension is lower than the other's rank). These observations raise a possibility that social comparisons play a crucial role in the modulation of self emotional states on empathy for others. Using functional MRI with an empathic paradigm, the current project aims to elucidate the mechanisms underlying the influence of self emotions on empathic responses in social comparison contexts. We found that in upward social comparison contexts, participants exhibited reduced positive empathic responses toward confederates' positive outcomes. This was mirrored by significant activation in the aIC and dorsal anterior cingulate cortex. Moreover, the decrease in positive empathic responses was significantly correlated with the self-reported envy ratings, with the aIC activity encoding individual envy ratings. Compared with the baseline positive empathic responses, the strength of functional connectivity from the aIC to the brain area involved in the processing of positive states of others (i.e., ventromedial prefrontal cortex and striatum) scaled with the reduction in positive empathic responses. Taken together, we conclude that an individual's negative social emotion (envy) elicited by upward social comparisons underpins the blunted positive empathic responses, which involves the functional interaction between brain regions processing upward social comparisons (aIC) and positive empathy (ventromedial prefrontal cortex and striatum). These findings not only enhance our understanding about the neural basis of human empathy, but provide insights into the pathogenic mechanisms in relevant neuropsychiatric disorders.

Title of Project: Dissect Cerebellar Mechanism and Therapeutics of Tremor Subtypes by Spatio-Temporal Neural Dynamics

Project No.: NHRI-EX113-11303NI
P.I. Name: Ming-Kai Pan/潘明楷
Key Professional Personnel: Wen-Chuan Liu
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2024 to 2027 (Total: 4 years)

Essential tremor (ET) is the most common movement disorder, characterized primarily by action tremor. Despite its prevalence, therapeutic responses are varied and often unsatisfactory, indicating multiple underlying pathophysiologies for different ET subtypes. Less than 50% of ET patients respond to pharmacological therapy, and no new medications have been developed in the past 25 years. From 2023 to 2024, six pharmaceutical companies reported unsuccessful clinical trials for ET medications, collectively spending over one billion US dollars. This highlights the urgent unmet medical need for effective ET therapies and a new understanding of the pathophysiology underlying drug-refractory ET.

In this project, we aim to elucidate two potential mechanisms that could explain the dichotomy in drug responses. Our primary objective is to uncover the circuitry dynamics that underlie the pathophysiology of each ET subtype. Additionally, we aim to design novel therapeutic approaches based on the underlying pathophysiology of drug-refractory ET subtypes. We utilized two mouse models for ET: the harmaline-induced tremor model, which facilitated the discovery of propranolol and primidone 25 years ago, and the *Grid2*^{dupE3} mouse model identified by our group, which exhibits ET-like climbing fiber (CF) overgrowth in the cerebellum (*Science Translational Medicine*, 2020).

In the first year, we observed that harmaline-induced tremors responded to both propranolol and primidone, while tremors in *Grid2*^{dupE3} mice were refractory to both medications. We then investigated whether the two models encode tremor frequency through distinct mechanisms. Both models exhibited similar cerebellar population activity for frequency coding, which did not explain their diverse pharmacological responses. Notably, tremor frequencies were approximately 13 Hz in harmaline-treated mice and 20 Hz in *Grid2*^{dupE3} mice, suggesting that higher tremor frequency might contribute to the drug-refractory state. However, a subset of 17 Hz tremors in harmaline-treated mice remained drug-responsive, while *Grid2*^{dupE3} mice remained drug-refractory, indicating that cerebellar frequency coding for tremors is not a contributing factor distinguishing drug-responsive from drug-refractory ET subtypes. In the following years, we will investigate the inter-neuronal synchrony and its contribution to drug refractory in ET.

The first year's work also helped to confirm the general principle of tremor frequency coding, marking the first numerically precise mechanism for neural dynamic fields and introducing a physics-like approach to biology. These findings are published in *Science Translational Medicine*, 2024.

Title of Project: Mechanisms and Intervention for Vascular Cognitive Impairment - Microglia Mediated Neuro - inflammation in Cognitive Impairment After Ischemic Brain Injury

Project No.: NHRI-EX113-11132HT
R.I. Name: JoenRong Sheu/許準榕
NHRI Researcher: Hung-Yi Chiou/邱弘毅
Key Professional Personnel: Joen-Rong Sheu/許準榕, Chih-Hao Yang/楊志豪
Affiliation/Institution: Taipei Medical University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Microglia-mediated neuro-inflammation plays a significant role in the dysfunction of the glymphatic system, a crucial pathway for clearing waste products from the brain. This dysfunction could pathologically contribute to the cognitive impairment following ischemic brain injury, including conditions like Alzheimer's and Parkinson's disease. Utilizing photothrombotic stroke models combined with tPA-lytic treatment and chronic cerebral hypoperfusion models, we identified key interactions between hyper-reactive microglia with glymphatic structures. Our findings highlighted the impairment of glymphatic clearance, decreased astrocytic endfeet coverage on blood vessels, and reactive microglial infiltration into the perivascular space post-stroke. Chemogenetic suppression of hyper-reactive microglia preserved glymphatic function and improved cognitive outcomes, indicating a direct link between neuro-inflammation and glymphatic dysfunction. Our focus then shifted to the molecular mechanisms underlying this dysfunction. Through the combination of stroke models with rehabilitation therapy and microglia-specific transcriptomic analyses, we identified TRIM5 as a crucial mediator. Among the TRIM family proteins, TRIM5 exhibited the most prominent regulation, localizing to microglial cells interacting with glymphatic structures. Using TRIM5 knockout mice, we demonstrated that the absence of TRIM5 ameliorated stroke-induced motor, cognitive, and emotional impairments, underscoring its pathological role. Our research provides direct evidence of the pathological role of microglia-mediated neuro-inflammation in glymphatic dysfunction following ischemic brain injury. Understanding these mechanisms offers valuable therapeutic targets for improving long-term cognitive outcomes in stroke patients.

Title of Project: Targeting ALS by a Novel Conserved Motor Neuron Micropeptide Derived from LncRNA

Project No.: NHRI-EX113-11330NI
P.I. Name: Jun-An Chen/陳俊安
Key Professional Personnel: Fang-Yu Hsu/許芳瑜
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2024 to 2026 (Total: 3 years)

The presence of small open reading frame (smORF)-encoded micropeptides within long non-coding RNA (lncRNA) regions is often underappreciated due to their limited size and scarcity. However, emerging evidence has shed light on their roles in fundamental biological processes, although their contribution to neural development and neurodegeneration remains unclear. To address this knowledge gap, we used spinal motor neurons (MNs) as a paradigm to investigate the function of a murine micropeptide, Sertm2, which is encoded by the lncRNA *A730046J19Rik*, during MN development. Our preliminary results indicate that this micropeptide is a highly conserved putative transmembrane protein expressed abundantly in postmitotic MNs. Interestingly, genetic deletion of *A730046J19Rik* from MN subtypes induced retrograde signaling of muscle neurotrophic Glial cell line-derived neurotrophic factor (GDNF), revealing that the Sertm2 micropeptide may modulate this signaling pathway. In addition, our experiments on a mouse model of ALS support that reducing *A730046J19Rik* levels by genetic ablation could significantly extend the lifespan of affected mice, indicating the potential for *Sertm2/A730046J19Rik* as a new therapeutic target for neurodegenerative diseases such as ALS. Therefore, we systematically identify micropeptides in spinal MNs, verify their expression *in vivo*, and then use *Sertm2/A730046J19Rik* as a paradigm to elucidate the role of lncRNA-derived micropeptides in neural development and neurodegeneration. Collectively, our results may provide the first comprehensive blueprint of the micropeptidome in spinal MNs and open up new avenues for targeting micropeptides as potential therapeutics against neurodegenerative diseases.

Title : Impacts of SARS-CoV-2 Structure Proteins on the Peripheral Sensory Neurons in *Drosophila melanogaster*

P.I. Name : Han-Hsuan Liu/劉翰璇

Presenter : Tzu-Hsuan Huang, Min-Hsien Wang, and Han-Hsuan Liu

Institute/Center : Center for Neuropsychiatric Research, National Health Research Institutes

Over 600 million people worldwide have been infected with SARS-CoV-2. While many patients experience acute symptoms, some patients suffer from prolonged effects such as brain fog, post-exertional malaise, and neuropathic pain. Unfortunately, the mechanisms by which this respiratory virus impacts the nervous system remain largely unknown. In our study, we explore the causes of neuropathic pain in SARS-CoV-2 patients using the *Drosophila* peripheral sensory neurons- class 4 dendrite arborization (c4da) neurons as our model. We analyzed the neuronal structures and functions of c4da neurons with 29 individual SARS-CoV-2 structural protein overexpression. Our results showed that excessive NSP5 in c4da neurons leads to lethality, and 11 of SARS-CoV-2 proteins disrupt dendrite development to varying extents. Further analysis revealed that the expression of 8 SARS-CoV-2 proteins in the c4da neurons that impaired dendrite structures also affected c4da neuron-mediated chemical nociception escape behavior. These results suggest that specific SARS-CoV-2 proteins negatively modulate the structure and function of fly c4da sensory neurons. To expand our understanding, we are examining dendrite defects in adult sensory neurons to determine if aging exacerbates these phenotypes. Additionally, we are using bioinformatic analysis to predict signaling pathways enriched with host proteins targeted by SARS-CoV-2 proteins. We aim to identify potential treatment targets or pathways to improve the quality of life for those affected by SARS-CoV-2.

Keywords: SARS-CoV-2, dendrite structure, and sensory neurons

Title of Project: Pharmacomicrobiomics Investigation and Gut Microbiome Analysis of Breast Cancer Patients Receiving Oral Medication
Project No.: NHRI-EX113-11003BC
P.I. Name: Cheng-Chih Richard Hsu/徐丞志
NHRI Researcher: Mingzi M. Zhang/張明姿
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2021 to 2024 (Total: 4 years)

Drug responses can vary greatly among individual patients in many diseases, leading to compromised drug efficacy, side effects, and even death of patients. In the past few years, pharmacomicrobiomics research has revealed a possible explanation that human gut microbes are involved in the metabolism of drugs, influencing therapeutic outcomes, such as drug efficacy and toxicity. Despite this understanding, our knowledge of gut microbiome-drug interactions remains limited. Many diseases still have not yet been explored, which could hamper the development of personalized treatments and potentially lead to tremendous costs in wasting clinical resources. Breast cancer with a large population (the second most common cancer worldwide) and with existing inter-patient variety in drug responses is highly worthy but yet to be studied. Therefore, we collaborated with the breast cancer research team at National Taiwan University Hospital (NTUH) to initiate a longitudinal case-cohort of breast cancer HR+ patients taking oral anti-cancer medication, including the two most common endocrine therapy drugs (letrozole, tamoxifen) and three newly approved target therapy CDK 4/6 inhibitors (palbociclib, ribociclib, and abemaciclib). We developed several bioanalytical tools including drug-gut microbe interaction *in vitro* and *ex vivo* assays to determine which gut microbes were capable of metabolizing target drugs, targeted quantification mass spectrometry method by triple quadrupole mass spectrometer (QTRAP 5500 LC-MS/MS System, SCIEX), untargeted screening mass spectrometry method by high-resolution orbitrap mass spectrometer (Q-Exactive Plus LC-MS/MS System, Thermo Scientific), and genome editing tools using CRISPR-Cas9 technology which enabled enhanced transformation efficiency in *Bifidobacterium* and successful base editing. The quantification platforms were successfully validated, exhibiting a wide linear range, high accuracy, precision, recovery rate, and minimal matrix effect. In the cohort study, we enrolled a total of 200 HR+ breast cancer patients. We identified the gut microbiome composition of 142 patients by next-generation sequencing analysis and we found that the gut microbiome compositions of these 142 breast cancer patients were highly individualized and yet less dependent on drug administration by principal co-ordinates analysis (PCoA). Also, we determined the pharmacokinetic outcome by quantifying the steady-state serum drug concentration of 156 patients by targeted mass spectrometry analysis, which showed the inter-patient variability in steady-state serum concentration. By performing Spearman's rank correlation analysis, we successfully identified several key gut microbial species which were associated with changes in pharmacokinetic outcome, suggesting these gut microbes might play a role in modulating pharmacokinetics by their drug-metabolizing activity. Overall, we have applied the untargeted and targeted mass spectrometry methods and next-generation sequencing analysis to quantify BC patient serum drug concentrations and study gut microbe-drug interactions, as well as developed the genome-editing technology using CRISPR-Cas9 system.

Title of Project: Functional Analysis of a Novel RCC-associated Macrophage Subpopulation at the Single-cell Level

Project No.: NHRI-EX113-11101BI

P.I. Name: Tien Hsu/徐沺

Key Professional Personnel: Thi-Ngoc Nguyen/阮氏玉, Heu-Huy Nguyen-Tran/阮陳孝輝, Tien Hsu/徐沺

Affiliation/Institution: China Medical University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Lipid-laden Macrophages in ccRCC Development: How They Are Formed and How They Contribute to Tumorigenesis

Renal cell carcinoma (RCC) is the most common form of kidney cancer. Most RCC patients present notable symptoms (hematuria, anemia, cachexia, and flank pain) only in advanced stages, making early detection difficult. The majority (>60%) of RCC cases are diagnosed incidentally by noninvasive imaging, and up to 50% of the cases are diagnosed at metastatic stages. Clear-cell renal cell carcinoma (ccRCC) constitutes the majority (nearly 80%) of primary RCC. Notably, while the 5-year survival rate of early-stage ccRCC can be up to 90%, that of metastatic ccRCC is only about 10%. These statistics point to the need for early detection and treatment of ccRCC.

ccRCC is characterized by the appearance of cancer cells with lipid-filled cytoplasm, hence the name "clear-cell". Excessive lipid accumulation is often regarded as a biproduct of metabolic abnormalities, but in fact stored lipids serve critical functions in pathological conditions such as cancers. Stored lipids not only provide energy reserves for tumor growth and cell movements but also supply raw materials for membrane construction during rapid cell proliferation. In addition, stored lipids also serve an important function for reducing cellular lipotoxic stress (from peroxidized fatty acids) resulting from excessive reactive oxygen species (ROS). Thus many cancers, exemplified by ccRCC, accumulate lipids in the form of lipid droplets. It has been assumed that increased lipid contents in ccRCC and other cancer cells are the result of defective oxidative phosphorylation in metabolism, leading to "reverse TCA (tricarboxylic acid) cycle" that overproduces acetyl-CoA for lipid synthesis. However, it has long been a puzzle that ccRCC cancer cells when cultured *in vitro* rarely exhibit prominent clear-cell phenotype, although they do become clear-cell tumor once implanted in the xenograft animal model, suggesting the importance of microenvironment in the process.

Genetic and epigenetic inactivation of the *VHL* tumor suppressor gene is the major genomic defect in a great majority of ccRCC cases. During our studies of the macrophages (M s) associated with ccRCC, we noticed a significant increase in genes related to lipid accumulation in the M s cocultured with *VHL*-deficient kidney tubule cells. Subsequent analysis confirmed that these tumor-associated M s (TAMs) could indeed uptake lipids and become lipid-laden M s (LLMs), and these LLMs can then transfer the lipid contents to kidney tubule cells via specialized cellular structures called tunneling nanotubes (TNTs), raising the possibility that formation of the clear-cell phenotype may be aided by LLMs *in vivo*, thus promoting ccRCC tumorigenesis. We will systematically study these highly interesting findings, which we believe will provide significant novel insights into the oncogenic process of ccRCC and other metabolically abnormal malignancies, and suggest new therapeutic and diagnostic strategies.

Title of Project: Analysis of Plasma Membrane Injury Elicited Tumor-derived Extracellular Vesicles
Project No.: NHRI-EX113-11102BI
P.I. Name: Wei Yuan Yang/楊維元
Key Professional Personnel: Hsiang-Yi Chang/張項詒, Hung Chang/張閎
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2022 to 2024 (Total: 3 years)

The goal of this proposal is to examine how plasma membrane injury (PM-injury) elicits tumor-derived extracellular vesicle (TEV) formation to carry out signaling functions. Our findings suggest that TEVs are generated primarily through two parallel mechanisms. First, through Cryo-ET, we found that cells are capable of generating actin-filled protrusions to mediate the release of TEVs through outward budding. Second, through CLEM and the MVB reporter pHluorin-CD63, we identified amphisome production as a route for enhanced TEV release following PM injury. We found that the transcription factor TFEB is activated during PM injury, potentially facilitating TEV production via these two pathways. Additionally, we identified cellular conditions that can route materials to be released through TEVs by the amphisome pathway toward intracellular degradation by the autophagy pathway. This strategy may potentially be used to alter TEV profiles for anti-tumor therapy.

Title of Project: Metagenomics Analysis and Bacteria-targeted Phage Therapy in Intestinal Carcinogenesis

Project No.: NHRI-EX113-11108BI
P.I. Name: Linda Chia-Hui Yu/余佳慧
Key Professional Personnel: Liang-Chuan Lai/賴亮全, Shu-Chen Wei/魏淑鉁, Yen-Hsuan Ni/倪衍玄
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Accumulating evidence indicates that besides gene mutation, altered microbiota played a causative role in intestinal cancer development. Our previous NHRI project demonstrated that microbiota dysbiosis correlated with altered antimicrobial transcriptomes in a mouse model of chemical-induced cancers. An *E. coli* strain isolated from colonocytes promoted epithelial hyperproliferation and caused cancer initiation in the recipient mice, implicating its role as a tumorigenic invasive pathobiont. **Despite clear tumorigenic evidence of gut bacteria, unique gene signatures in the oncogenic pathobionts to distinguish from symbionts remain poorly defined and pathobiont-targeting therapy has not been developed.** Recently, altered virome was shown in many diseases and implicated that bacteriophages may impact tumor progression. To date, protumoral genes in the pathobionts remain elusive and the anticancer potential of bacteriophage therapy targeting pathobionts awaits to be explored. **The overall aim is to investigate the host-microbiome interaction in colorectal carcinogenesis using metagenomics approaches and exploration of bacteriophage therapy.** The genetic mutant Apc(Min/+) mice showed multiple tumors in the intestines by 20 weeks old, of which the tumor numbers and area were reduced by timely antibiotic (ABX) treatment at 8-10 weeks of age. The presence of intraepithelial bacteria was also observed in the intestines of Apc(Min/+) mice at 8 and 20 week-old, showing enrichment of *Escherichia* inside epithelial cells as determined by 16S rDNA sequencing. Segregation of epithelial microbiota was noted before that of fecal microbiota between Apc(Min/+) and WT (+/+) littermates, whereby a higher relative abundance of *Escherichia* and *Akkermansia* was found in the Apc(Min/+) mice. Inoculation with invasive *E. coli* LI60C3 strain, but not virulence-deleted ΔHtrA, increased tumor burden and spheroid growth of Apc(Min/+) mice. Increased spheroid formation and elevated epithelia-derived free radical production were associated with higher cancer stemness CD44 expression in gut epithelial cells of Apc(Min/+) mice after LI60C3 inoculation. The *in vitro* studies showed that bacterial invasion led to elevated levels of CD44 variant 3 (v3) and variant 6 (v6) and Hippo signaling pathways in human intestinal epithelial Caco-2 cells. Bacterial infection also upregulated luciferase-driven CD44 promoter activity, providing direct evidence of microbe-induced CD44 upregulation independent of immune cells. The tumorigenic invasive *E. coli* was subjected to whole genome sequencing by using PacBio Sequel II platforms and revealed a circular chromosome of 4,863,930 bases accompanied by two endogenous plasmids (i.e., 81809 and 8556 bases). By comparing the whole genome to other *E. coli* strains published in the GeneBank database, we found that the invasive LI60C3 belongs to the B2 phylogroup and harbors unique pathogenicity islands. The unique bacterial genetic signatures were used for validation in human CRC specimens which showed increased expression compared with healthy controls. Moreover, a new phage targeting LI60C3 was isolated from the natural environment and demonstrated a strong bacteriolytic ability. Our pilot study has demonstrated that treatment with the newly identified lytic phage exerted tumor reduction in preclinical mouse models. Lastly, virus-like particles (VLP) were prepared from fecal samples with minimal contamination of mouse *Gapdh* and bacterial 16S genes. Virome analysis was performed using *de novo* assembly and protein-coding gene prediction for virus taxonomy. Cluster analysis showed the presence of *Caudoviricetes, Siphovirus, Podovirus,* and *Escherichia* phage in CRC feces. In sum, our study will provide novel insights into host-microbe interaction underlying intestinal tumorigenesis that involves three-way crosstalks between epithelia, bacteriome, and virome.

Title of Project: Molecular and Therapeutic Significance of ZBTB46-PCK1 in Prostate Cancer
Project No.: NHRI-EX113-11109BI
P.I. Name: Yen-Nien Liu/劉晏年
Key Professional Personnel: Yen-Nien Liu/劉晏年
Affiliation/Institution: Taipei Medical University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

The intricate interplay between prostate stromal cells and prostate cancer (PCa) cells is critical for promoting tumor growth and immune evasion within the tumor microenvironment (TME). Our research uncovers the pivotal role of the leukemia inhibitory factor (LIF)/LIF receptor (LIFR) pathway in mediating these effects, particularly under conditions of androgen deprivation therapy (ADT). We demonstrate that activation of LIF/LIFR signaling in both prostate tumor and stromal cells fosters an immunosuppressive TME. Specifically, LIF/LIFR activation in PCa cells drives neuroendocrine differentiation (NED) and upregulates immune checkpoint molecules, while inhibition of this pathway significantly mitigates these effects, highlighting its central role in linking NED to immunosuppression. Furthermore, prostate stromal cells expressing LIFR amplify NED and immunosuppressive markers in PCa cells, a process reversible by LIFR knockdown. Notably, ADT-induced LIF/LIFR signaling enhances brain-derived neurotrophic factor (BDNF) expression, which subsequently promotes NED, tumor aggressiveness, and immune evasion in PCa cells. Clinical data corroborate these findings, revealing elevated BDNF levels in metastatic castration-resistant PCa (CRPC) and a strong correlation with programmed death-ligand 1 (PD-L1) and other immunosuppressive markers. Our study elucidates the significant crosstalk between PCa cells and prostate stromal cells, emphasizing the enhancement of LIF/LIFR signaling as a driver of an immunosuppressive TME and NED in PCa cells through BDNF upregulation. These insights present potential therapeutic targets for disrupting the pro-tumorigenic and immunosuppressive dynamics within the prostate TME.

Title of Project: Study of the Cancer Associated VLDL and VLDLR Roles in Hepatocellular Carcinoma
Project No.: NHRI-EX113-11110BI
P.I. Name: Wen-Lung Ma/馬文隆
Affiliation/Institution: China Medical University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Optimization of VLDL-mimicking Lipid-polymer Hybrid Nanoparticles (VMND) for Hepatocellular Carcinoma Treatment

Except for curative surgical removal of tumor lesions, clinical management for advanced Hepatocellular carcinoma (HCC) using multi-kinase inhibitors, e.g., Lenvatinib, as 1st line targeted therapy. Yet, the marginal survival benefit of Lenvatinib definitely can be improved from low response rate in patients. There are two main strategies to increase response rate in drug design. One is precisely selecting patients with theragnostic markers, the other is improving drug distribution with targeted control-release formulation. Recently, our team has discovered HCC prognosis related lipid metabolism events. 1. We found VLDLR overly expressed in tumor lesion, suggesting a VLDL addictive behavior of HCC. Just like the Helen of Troy welcoming the Trojan Horse. 2. There are also lipid metabolites favorable for good prognosis. Take the advantage of those discoveries, the current proposal would like to translate them as one pill. The first step is to realize the idea of VLDL mimicking nanoparticle drug carrying Lenvatinib (VMND) as therapeutics. The second step is to test the possibility of lipidomic-based VMND drug design. For the first step, we have been practiced it with a proof-of-concept experiment performed by the team. Hence, this proposal would like to define manufacture procedure for future scale-up. Lipid-polymer hybrid nanoparticle delivery systems have combined numerous advantages including the polymeric core and the biomimetic ability of the phospholipid shell into a single platform. VMND comprising a poly (lactic-co-glycolic acid) (PLGA) core and a lipid shell composed of 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, TPGS, and 1,2-disteroyl-sn-glycero-3- phosphoethanolamine N [succinyl-polyethylene glycol] (DSPE-PEG 2000) synthesized to improve the therapeutic potential of Lenvatinib through novel engineered delivery system VMND. With successfully establishment of lipid–polymer hybrid nanoparticle delivery systems in the lab, we're able to stabilized manufacture procedure in the team. For the second step, we will be optimizing the lipid loading capacity with add-in prognostic favorable lipid metabolites in the VMND manufacturing process. With the success in step one, the second step will be more detailed in defining lipid composition and formulation optimization.

Title of Project: The Impacts of Diphthamide Modification of Eukaryotic Elongation Factor 2 (eEF2) in Hepatocellular Carcinogenesis

Project No.: NHRI-EX113-11122BI

P.I. Name: Chun-Ming Chen/陳俊銘

Key Professional Personnel: Pin-Huang Lee/李品宏, Feng-Chi Chiu/丘鳳棋

Affiliation/Institution: National Yang Ming Chiao Tung University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Diphthamide is a crucial post-translational modification on the histidine residue of eukaryotic elongation factor 2 (eEF2), assuring the fidelity of mRNA translation and regulating translation efficiency. To investigate the impact of diphthamide in hepatocellular carcinoma (HCC), we previously demonstrated that *Dph1*-deficient livers exhibited reduced DEN-induced liver tumorigenesis by suppressing compensatory proliferation and promoting cellular death. However, in the context of *Pten/Trp53*-deficient HCC, *Dph1* deficiency in the liver promoted tumorigenesis by increasing the population of K19+ or CD133+ cells. These findings indicate that the loss of *Dph1* and subsequent diphthamide deficiency plays a context-specific role in hepatocellular carcinogenesis. Furthermore, we investigated the effectiveness of sorafenib treatment in liver tumor organoids derived from hepatocyte-specific *Pten/Trp53*- and *Pten/Trp53/Dph1*-knockout mice. Interestingly, our findings revealed that *Pten/Trp53/Dph1*-deficient liver tumor organoids exhibited resistance to sorafenib, which correlated with enhanced stem/progenitor characteristics. Additionally, differential levels of endogenous mitochondrial reactive oxygen species have been linked to sorafenib responsiveness in *Pten/Trp53*- and *Pten/Trp53/Dph1*-knockout HCC organoids. The sorafenib resistance observed in *Pten/Trp53/Dph1*-deficient liver tumor organoids will be investigated further to understand the role of unmodified eEF2 in the underlying resistance mechanism.

Title of Project: Elucidating the Function Roles of Pancreatic Cancer-derived Exosomes in Fat Loss
Project No.: NHRI-EX113-11123BI
P.I. Name: Chun-Mei Hu/胡春美
Key Professional Personnel: Sui-Chih Tien/田穗稡
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Over 80% of the patients with pancreatic ductal adenocarcinoma (PDAC) have cachexia/wasting syndrome. Cachexia is associated with reduced survival, decreased quality of life, and higher metastasis rates. Here, we demonstrate that fat loss is the earliest feature of PDAC–exosome-induced cachexia. MicroRNA sequencing of exosomal components from normal and cancer-derived exosomes revealed enrichment of miR-16-5p, miR-21-5p, miR-29a-3p, and miR-125b-5p in serum exosomes of mice harboring PDAC and patients with PDAC. Further, miR-16-5p and miR-29a-3p inhibited adipogenesis through decreasing Erlin2 and Cmpk1 expression which downregulates C/EBP and PPAR . Synergistically, miR-29a-3p promotes lipolysis through increasing ATGL expression by suppressing MCT1 expression. Furthermore, PDAC–exosomes deprived of miR-16-5p and miR-29a-3p fail to induce fat loss. Hence, miR-16-5p and miR-29a-3p exosomal miRs are essential for PDAC-induced fat loss. Thus, we unravel that PDAC induces adipose atrophy via exosomal miRs. This knowledge may provide new diagnostic and therapeutic strategies for PDAC-induced cachexia.

Title of Project: KIF2C as a Novel Therapeutic Target of Breast Cancer
Project No.: NHRI-EX113-11124BI
P.I. Name: LilyHui-Ching Wang/王慧菁
NHRI Researcher: Ching-Chuan Kuo/郭靜娟
Affiliation/Institution: National Tsing Hua University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

In this project, we aim to explore KIF2C, a microtubule depolymerizer in mitosis, as a druggable therapeutic target to fight against chemoresistance of breast cancer. Our ultimate goal is to develop a novel KIF2Ci as a first-in-class therapeutics of breast cancer. In the past 2.5 years, we have accomplished the following achievements (currently in revision) :

1. **Characterize the molecular interplay between KIF2C and paclitaxel-stabilized microtubule dynamics**: We successfully produced recombinant active KIF2C and accomplished the *in vitro* functional characterization regarding the molecular mechanism in which KIF2C catalyzes the resolution stabilized by paclitaxel. This serves the ground basis mechanism to explain the development of KIF2C-mediated chemoresistance in breast cancer.
2. **Characterize tubulin isotype composition and post-translational modification in paclitaxel resistant TNBC:** Here we clarified the tubulin isotype composition using an LC-MS/MS approach and found that tubulin β4a was increased in MDA-MB-231 R cells. We find that tubulin isotype composition may play a role in the development of chemoresistance in MDA-MB-231 cells, but not in 4T1 cells. In addition, both tubulin tyrosination and polyglutamylation were increased in chemoresistant 4T1 cells and we found that overexpression of carboxypeptidases VASH1/2-SVBP, which catalyzed tubulin detyrosination, blocked TTLL4-mediated tubulin polyglutamylation. Next, polyglutamylated peptides were detected only on tyrosinated α tubulin in our LC-MS/MS analysis. Thus, tubulin tyrosination is likely a prerequisite of subsequent polyglutamylation.
3. **Comparison with currently available and our developed KIF2C inhibitors**: Before this study, two KIF2C inhibitors had been reported: DHTP and C4. DHTP functions as an allosteric inhibitor of the kinesin 13 family. We confirmed that DHTP inhibited kinesin activities of KIF2A, KIF2B, and KIF2C. However, the cell-penetrating ability of DHTP was significantly lower than that of 7S9, posing challenges for its future applications. Another KIF2C inhibitor, C4,9 did not inhibit KIF2C activity in our kinesin assay but did induce cytotoxicity, albeit at high molar concentrations. Here we show that 7S9 is the most potent KIF2C inhibitor, with the lowest IC50 value. Depletion of KIF2C abolished 7S9-mediated cytotoxicity. In addition, 7S9 selectively inhibited KIF2C activity, whereas DHTP inhibited the kinesin-13 family. Taken together, our results indicated that 7S9 is the most potent, selective, and cell-penetrating inhibitor of KIF2C.

Title of Project: Investigation of the Mechanism and Medical Implications of a Novel Histone Lysine Demethylase KDM4A Interacting Long Non-coding RNAs (LncRNAs) LINC01061 in Viral Reactivation and Tumorigenesis

Project No.: NHRI-EX113-11125BI
P.I. Name: Pei-Ching Chang/張佩靖
Key Professional Personnel: Pei-Ching Chang/張佩靖
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

The impact of histone lysine demethylases 4A (KDM4A), also known as JMJD2A, on epigenetic reprograming has long been investigated, focusing on H3K9me3, a heterochromatin mark. In general, removal of H3K9me3 from a promoter region by KDM4A is associated with gene up-regulation. However, when complexed with histone deacetylases (HDACs), KDM4A removes acetylation from the promoter region, leading to gene repression. These data indicate that KDM4A may either activate or repress transcription depending on its interacting partners. More importantly, in oocytes, KDM4A regulates the maternal-to-zygotic transition not only by demethylating H3K9me3 in broad domains of H3K4me3 (bdH3K4me3) but also by protecting these domains from invasion by H3K9me3. This protection is critical for chromatin opening and gene transactivation during embryo development. However, little is known about the relocation of KDM4A from promoter region to barrier sites. Here, using Kaposi's sarcoma associated herpesvirus (KSHV) as a screening model, we identified 6 oncogenic virus-induced long non-coding RNAs (lncRNAs) with the potential to open chromatin. RNA immunoprecipitation (RIP) revealed KSHV-induced LINC01061, we named it KDM4A-associated transcript (KIKAT), as a KDM4A binding partner. Integrated chromatin immunoprecipitation sequencing (ChIP-seq) and RNA-seq analysis showed that the KIKAT interaction with KDM4A may mediate the relocating of KDM4A from the transcription start site (TSS) and transactivation of genes. Thus, we conclude that KIKAT triggered the shift of KDM4A as a potential epigenetic mechanism that regulates gene transactivation. The breakdown of the interaction of KIKAT and KDM4A may represent a novel therapeutic mechanism that inhibits the oncogenicity of KDM4A.

Title of Project: Circulating Tumor Cell-derived Organoid on-a-chip: Applications for Colorectal Cancer Drug Discovery

Project No.: NHRI-EX113-11126BI
P.I. Name: Fan-Gang Tseng/曾繁根
Key Professional Personnel: Long-Sheng Lu/呂隆昇
Affiliation/Institution: National Tsing Hua University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Cancer is one of the top causes of death in Taiwan, where the critical factor leading to cancer-related mortality is metastasis. Metastatic cancer cells may experience the intravasation process transforming into circulating tumor cells (CTCs) to form new tumors in secondary sites. This kind of distant cell migration drives cancer progression, leading to patient death. This process of "intravasation" is a crucial step in the metastasis of primary tumors, where cancer cells invade the stroma through complex bidirectional signaling to further penetrate microvessels and enter the circulatory system. By studying the special *in vitro* microenvironments, we could know more about the tendency of cancer cell metastasis, aiming to develop more effective treatments. This research aims to establish a platform based on a novel chip-based biomimetic microenvironment model in collaboration with a chip-array fluidic system for tumor-on-a-chip study. Previously, our team has developed biomimetic tumor microenvironment platforms based on Alginate Hydrogel Tube (AHT) and Transwell-based Alginate Hydrogel (TAH). By those, the microenvironment of ovarian cancer metastasis to the peritoneum were investigated, and successfully simulated the mechanism of distant metastasis of cancer cells. Building upon our prior studies, we further develop a completely new biomimetic tumor microenvironment (TME) platform to address the issue of instability in AHT and TAH platforms. This platform integrates the Cell-Separation Dynamic-Staining Self-Assembly Cell Array (CSDS-SACA) Chip with the chip-array fluid device, offering a novel approach to enhance experimental stability, consistency, and significantly improve research efficiency. The platform consists of our microfluidic chip, 3D-printed scaffolds with flow channels, a peristaltic pump, and tubing. The scaffold is designed with a space to accommodate the CSDS-SACA chip, which is secured by clamps or screws and nuts. The CSDS-SACA chip is an independent chip with a porous membrane at the bottom and an upper opening. Based on this design, individual cells and co-cultured cells could be both cultured on this chip to provide more adjustable factors for TME simulation. Once the assembled chip and scaffold are connected to the micro-volume peristaltic pump and a medium reservoir, the platform is completely set up. Through this microfluidic chip-array platform, we have significantly enhanced the stability of experiments by replacing the previous design using hydrogel tubes. Additionally, this platform allows multiple chips to be applied simultaneously to perform multiple experiments at the same time, thereby significantly improving experimental efficiency. HT29 cells were cultured on the top of membrane in CSDS-SACA chip to mimic tumor tissue in the human body, while HUVEC cells were cultured beneath the membrane to simulate the microvascular wall in the established TME platform, allowing us to replicate the homing and intravasation behaviors of tumor cells. In the intravasation experiments, we analyzed the culture medium collected beneath the membrane and successfully observed cells crossing from the porous membrane into the underlying culture medium. We also conducted experiments to investigate the intravasation of cancer cells under different serum concentrations, and under both dynamic, and static conditions. The established biomimetic circulatory platform provides an efficient 3D *in vitro* TME model for studying cancer metastasis. It has demonstrated the ability to create a more authentic tumor microenvironment suitable for investigating the intravasation preferences of different cancer cell lines. By adjusting various parameters such as the chemical gradients of biological factors within the circulation system, static or dynamic nature of the system and different flow rates, we can explore a broader range of research avenues in the future.

Title of Project: The Tumor-progression Function, Molecular Mechanism, and Targeting Potential of lncRNA Smyca

Project No.: NHRI-EX113-11127BI
P.I. Name: Ruey-Hwa Chen/陳瑞華
Key Professional Personnel: Han-Hsiun Chen/陳漢勳, Keng-Hao Chang/張耕豪
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Triple-negative breast cancer (TNBC) is the most devastating subtype of breast cancer owing to the aggressiveness and lacking therapeutic targets. Our previous study identified a novel lncRNA *Smyca*, which is highly expressed in TNBC to promote multiple malignant features. Here, we show that *Smyca* knockdown inhibits DNA double-strand break repair via error-free homologous recombination (HR) but not error-prone non-homologous end joining to mimic a BRCAness phenotype. Mechanistically, *Smyca* functions as a guide to facilitate the loading of FOXM1 transcription factor to the promoters of a set of HR genes, thereby promoting their expression. Consequently, *Smyca* knockdown aggravates genome instability caused by chemotherapeutic agents and PARP1 inhibitor Olaparib and promotes micronuclei formation for activating cGAS/STING innate immunity pathway and type I interferon (IFN) responses. Clinically, *Smyca* expression in TNBC correlates positively with therapy resistance and negatively with HR deficiency score, IFN signatures, and infiltration of anti-tumor immune cells. Our study identifies the dual roles of *Smyca* in creating a BRCAness phenotype and in activating cGAS/STING pathway in TNBC and suggests *Smyca* as a potential target for sensitizing TNBC to chemotherapy, PARP inhibitor, or immune therapy.

Title of Project: Mechanism of Tight Junction Protein ZO-1 Mediating Spindle Misorientation, Chromosomal Instability and Its Role in Colorectal Carcinogenesis
Project No.: NHRI-EX113-11204BC
P.I. Name: Wei-Ting Kuo/郭瑋庭
Key Professional Personnel: Wei-Ting Kuo/郭瑋庭
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2026 (Total: 4 years)

Background: One of the biggest health hazards to humans is colorectal cancer, which has limited treatments and substantial side effects despite improvements in genetic, inflammatory, and microbiome research that have helped us understand its progression. Recent study links abnormal tight junction (TJ) protein expression to tumor invasion and transformation in polarized epithelial cells. The specific effect of TJ protein ZO-1 on normal and altered intestinal epithelial cells is unknown, making therapeutic treatments challenging to develop. **Aim:** To explore the mechanisms by which ZO-1 mediates signals that directs mitotic spindle misorientation and chromosomal instability leading to tumorigenesis in the colorectal cancer. **Results:** A colitis-associated colon cancer model was created by delivering azoxymethane (AOM) and dextran sodium sulfate (DSS) to intestinal-specific ZO-1 knockout (ZO-1 KO^{IEC}) mice and their wild-type (WT) littermates of both genders. While there were no notable disparities in body weight and colon length between WT and ZO-1 KO^{IEC} mice, ZO-1 KO^{IEC} mice displayed increased tumor frequency and larger tumor sizes compared to WT mice. To analyze the molecular mechanism, CRISPR/Cas9 was used to construct ZO-1-deficient intestinal epithelial Caco-2 cells. It was shown that the cells did not have ZO-1 expression and had a weakened barrier function compared to WT cells. After treating with paclitaxel or nocodazole, ZO-1 KO cells experience increased apoptosis, which is the sub-G1 phase of cell cycle. In ZO-1 KO cells, a greater proportion of cells experience apoptosis and a misaligned mitotic spindle in 3D culture during mitosis, suggesting that the cell death caused by ZO-1 deficiency is a result of the mitotic spindle misorientation. Previous studies have shown that ZO-1 KO negatively impact the microvilli and cortical F-actin of epithelial cells, whereas ZO-1 interacts with the cortical F-actin of epithelial cells. By utilizing latrunculin A, cytochalasin B, jasplakinolide, and blebbistatin to disrupt actin polymerization and myosin motor function, it was shown that the influence of ZO-1 on cortical F-actin remained unchanged regardless of the orientation of the mitotic spindle. The RNAseq analysis conducted on the intestinal epithelial cells of ZO-1 KO^{IEC} mice revealed a significant alteration in genes related to mitosis. It is important to highlight that in ZO-1 KO Caco-2 cells, the downstream signal *YBX3* was increased, whereas the expression of *AURKA*, *CDK1*, *CDC25B*, and *ESPL1*, which are known to be involved in mitotic progression, was decreased. Furthermore, the expression of mitotic related apparatus and signals, such as Pericentrin and Aurora A, is reduced following the lack of ZO-1. ZO-1 may potentially engage in synergistic interactions with these genes to direct the movement of the spindle, consequently impacting DNA damage and chromosomal stability. In line with the hypothesized effect of ZO-1 deficiency, the study also found that ZO-1 deficiency was associated with increased DNA damage, which in turn led to chromosomal instability, as was demonstrated by the presence of phosphorylated H2AX, confirming ZO-1's role in regulating genomic stability. This effect was also observed in ZO-1 KO^{IEC} mice with AOM/DSS-induced colon cancer *in vivo*. The expression levels of *Cdk1*, *Tacc3*, *Espl1*, and *Cdc25b* were decreased, while the level of γH2AX was increased further suggesting the effect of ZO-1 deficiency in the contribution for cancer formation and progression. **Conclusions:** This work establishes that ZO-1 is crucial in the progression of colorectal cancer, since it governs the alignment of the mitotic spindle during cell division and influences cell death, chromosomal stability, and the proliferation of cancer cells. It also provides essential guidance for developing more effective and efficient treatment strategies in the future.

Title of Project: Exploiting PHF8-mediated Epigenetic Dependency in Gastric Cancer
Project No.: NHRI-EX113-11211BI
P.I. Name: Wen-Ching Wang/王雯靜
NHRI Researcher: Chiou-Hwa Yuh/喻秋華
Key Professional Personnel: Wen-Ching Wang/王雯靜, Chiou-Hwa Yuh/喻秋華, Ta-Sen Ye/葉大森, Ding-Jun Huang/黃鼎鈞, Chung-Yung Ma/馬崇勇
Affiliation/Institution: National Tsing Hua University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Deciphering the Role of PHF8/KDM7B in Gastric Cancer Progression and Therapeutic Intervention

We have identified PHF8/KDM7B as the most significant epigenetic KDM member associated with poor clinical outcomes in gastric cancer. Our study demonstrated that PHF8 co-activates JUN, thereby stimulating PKCα signaling, which is crucial for gastric cancer progression (Tzeng et al., PNAS 2020). Additionally, we solved the crystal structure of KDM4B (Chu et al., J. Med. Chem., 2014) and established that H. pylori infection triggers KDM4B-JUN-mediated epigenetic regulation, promoting inflammation and gastric cancer progression (Wu et al., Cell Death Dis., 2019). In this project, PI Wang (NTHU) collaborates with co-PI Yuh (NHRI; a zebrafish expert) and co-PI Yeh (CGMH; a gastrointestinal physician) to address the following aims: 1. Characterize the PHF8-JUN axis in regulating PTEN destabilization, building on and filling gaps in our preliminary results; 2. Investigate PHF8 as an epigenetic regulator of mitochondrial function; and 3. Dissect PHF8-mediated carcinogenesis in gastric cancer *in vivo*. PHF8 ChIP-seq analysis revealed its genomic binding near proximal promoters, influencing PTEN inactivation through the PHF8/c-Jun complex, enhancing PKCα expression, Src activation, and MKRN1-mediated PTEN degradation in gastric cancer. TEAD family transcription factors crucially regulate mitochondrial transcription, with preliminary data suggesting PHF8 as a TEAD4 co-activator in gastric cancer metabolism. Using transgenic fish overexpressed PKCa and RPIA in the intestine, to study combined PKCα, SRC, and MKRN1 inhibition effects. These strategies significantly reduced proliferation markers and trinucleated cells. Xenotransplantation experiments in zebrafish embryos confirm the synergistic efficacy of PKCα inhibitor Midostaurin, SRC inhibitor Bosutinib, shMKRN1, and a mitochondrial inhibitor in inhibiting gastric cancer proliferation and migration. Using the Phenotypic Response Surface (PRS) algorithm, we optimized dosages for xenotransplantation. We obtained the optimized dosages for combinational therapies via PRS and validated optimal drug concentrations significantly reduce cell viability by 50% around 12-16 hours in CCK8 assay. Moreover, after 12 hours of treatment with optimal concentrations of triple agents significantly reduced the MKN28-PLKO cell migration, while qPCR and JC-1 staining confirmed mitochondrial dysfunction. Our results suggest the potential of targeting the PHF8-JUN axis and related pathways as therapeutic strategies for combating gastric cancer. By elucidating the mechanisms by which PHF8 influences gastric cancer progression, our study provides a fundamental basis for the development of novel intervention strategies to improve patient outcomes.

Title of Project: Impact of PD-1 Post-translational Modifications and Trafficking in T Cells on Cancer Progression

Project No.: NHRI-EX113-11212BI
P.I. Name: Yi-Ching Wang/王憶卿
Key Professional Personnel: Yi-Ching Wang/王憶卿
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

To date, immune checkpoint inhibitor therapies targeting the programmed cell death-1 (PD-1) pathway have emerged as frontline treatments in cancer therapy. Nevertheless, our current understanding of PD-1-mediated regulation in T cells is still limited, underscoring the urgent need to gain a deeper insight into how PD-1 contributes to the process of T cell exhaustion and tumor immune escape. Our recent findings unravel novel mechanisms by which post-translational modifications (PTMs) such as phosphorylation, ubiquitarian, and glycosylation of PD-1 influence its stability, membrane presentation, and T-cell activity within the immunosuppressive tumor microenvironment. In addition, we identify novel mechanisms of intracellular trafficking and plasma membrane presentation of PD-1 mediated by Rab37 small GTPase to sustain T cell exhaustion leading to poor patient outcomes. PD-1 colocalizes with Rab37-specific vesicles of T cells in a GTP-dependent manner whereby Rab37 mediates dynamic trafficking and membrane presentation of PD-1. However, glycosylation mutant PD-1 delays cargo recruitment to the Rab37 vesicles, thus stalling membrane presentation. Our results also provide novel evidence that the IL-6/JAK2/USP24/PD-1 is an important regulatory pathway of PD-1 by which IL-6 activates JAK2-mediated PD-1 phosphorylation and USP24-mediated deubiquitination of PD-1 to prolong PD-1 protein stability and membrane presentation thereby decreases T cell functions. Moreover, we have developed therapeutic strategies targeting PD-1 PTMs using co-culture cell systems and syngeneic animal models. These strategies involve neutralizing antibodies, inhibitors, or our in-house developed antagonists targeting key enzymes identified in the PTM process. Clinically, the multiplex immunofluorescence-immunohistochemical assay indicated that cancer patients with high $Rab37^{+}/PD\text{-}1^{+}/TIM3^{+}/CD8^{+}$ tumor-infiltrating T cell signature or a high enrichment of aberrantly PTM-modified PD-1 in CD8 exhausted T cells correlate with advanced tumor stages and poor overall survival. Our results provide the first evidence of the role of Rab37 small GTPase in PD-1 trafficking and targeting IL-6/JAK2/USP24-mediated PTMs of PD-1 is a potential strategy for cancer treatment.

Title of Project: Targeting ER Protein TXNDC5 in the Tumor Stroma: Implications for Tumorigenesis and Therapy against Colorectal Cancer
Project No.: NHRI-EX113-11213BI
P.I. Name: Kai-Chien Yang/楊鎧鍵
Key Professional Personnel: Kai-Lin Cheng/程凱琳
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Targeting Tumor Stromal ER Protein TXNDC5 as Novel Therapeutic for Colorectal Cancer Treatment

Objectives: Mesenchymal-type colorectal cancer (CRC), characterized by strong stromal infiltration and immune tolerance, resists immune checkpoint blockade and has poor outcomes. Cancer-associated fibroblasts (CAFs), abundant in tumor stroma, actively remodel the extracellular matrix (ECM), aid immune evasion, and drive tumor progression. We have recently identified thioredoxin domain-containing protein 5 (TXNDC5), a protein disulfide isomerase (PDI), as a critical mediator of fibroblast activation and ECM remodeling in organ fibrosis. We hypothesized that TXNDC5 could also contribute to fibroblast activation, stroma formation and tumor progression in cancer, especially in the stroma-enriched fibrogenic mesenchymal-type CRC. Methods: Transcriptome databases of CRC were re-analyzed to determine the clinical relevance of TXNDC5. Experimentally, CRC was induced in mouse lines by azoxymethane (AOM) and dextran sulfate sodium (DSS) stimuli, a model sharing multiple characteristics with human mesenchymal-type CRC. Human colonic fibroblast line CCD-18co was used to investigate the molecular mechanisms by which TXNDC5 regulates colonic fibroblast activities. Fibroblast-specific TXNDC5 knockout (*Col1a2-Cre/ERT2*TXNDC5*$^{fl/fl}$, cKO) mice were generated, combining with single-cell RNA sequencing analyses on AOM/DSS-induced CRC tumors in these animals, to clarify how fibroblast TXNDC5 impact tumor microenvironment, CRC progression and response to immune checkpoint blockade. Findings: TXNDC5 was predominantly expressed in stromal fibroblasts of human and mouse CRC. Fibroblast-specific deletion of *Txndc5* lessened CAF activation, attenuated tumor fibrosis and reduced tumor burden in AOM/DSS-induced CRC. Mechanistically, increased TXNDC5 levels augments TGFβ signaling in CAF by post-translational stabilization of TGFBR1 through its PDI activity. In addition, deletion of *Txndc5* in CAFs led to less tumor desmoplasia, decompressed tumor vessels and attenuated intratumoral hypoxia, thereby easing immune tolerance and increasing cytotoxic T cell infiltration in CRC. Single-cell transcriptome analysis revealed a marked change of intratumoral immune cell populations upon fibroblast-specific deletion of TXNDC5, shifting from myeloid-derived suppressive cells to cytotoxic tumor-infiltrating lymphocytes. Importantly, depletion of TXNDC5 in CAFs potentiated the anti-tumor effects of immune checkpoint blockade with anti-PD1 therapy in CRC. Conclusions: Our data suggest an important yet previously unrecognized role of fibroblast TXNDC5 in CRC progression, through enhancing CAF activation, stroma formation and immune escape. Combining immune checkpoint blockade with TXNDC5 deletion synergistically improved anti-tumor effects in CRC. Targeting TXNDC5, therefore, can be a novel therapeutic approach for CRC patients.

Title of Project: Develop IL-19 Antibody Immunotherapy and Unravel Immunosuppressive Mechanism in Peritumoral Region of Glioblastoma by Single Cell Transcriptome Analysis

Project No.: NHRI-EX113-11214BI

P.I. Name: Cheng-Yu Chen/陳震宇

Key Professional Personnel: Gilbert Aaron Lee/李爾博, Yu-Wei Chang/張育維

Affiliation/Institution: Taipei Medical University

Entire Project Period: From 2023 to 2025 (Total: 3 years)

Glioblastoma multiforme (GBM) is a highly aggressive and resistant brain tumor, characterized by profound immunosuppression and chemoresistance, resulting in a dismal 5% survival rate at the five-year mark. Despite the application of standard therapies, which include a combination of surgery, radiotherapy, and temozolomide (TMZ) chemotherapy, tumors inevitably recur in the peritumoral region. Targeting multiple aspects of GBM-mediated immunosuppression and invasiveness is a potential therapeutic strategy to improve patient outcomes. In this study, we identified IL-19 as a predicted immunosuppressive cytokine in the peritumoral region, associated with poor survival in patients with GBM from both the TCGA and Taiwan GBM cohorts. Additionally, blocking IL-19 inhibits the progression of both TMZ-sensitive and TMZ-resistant tumors. Molecular studies revealed that IL-19 promotes TMZ-resistant GBM cell migration and invasion through a novel IL-19/WISP1 signaling pathway. Immunosuppressive M2 macrophage-derived IL-19 suppresses CD8 T cell activation. Taken together, IL-19 represents a therapeutic target to disrupt immunosuppressive responses in chemo-resistant GBM.

Title of Project: Deciphering the Mechanism and Clinical Significance of Cetuximab Resistance-mediated Microenvironmental Remodeling and Immune Checkpoint Inhibitor Resistance in Head and Neck Cancer

Project No.: NHRI-EX113-11215BI
P.I. Name: Muh-Hwa Yang/楊慕華
Key Professional Personnel: Po-Hsien Chiu/邱柏憲
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

The impact of targeted therapy on the subsequent use of immune checkpoint inhibitors (ICI) has been noted, but the underlying mechanisms remain largely elusive. In head and neck squamous cell carcinoma (HNSCC), subgroup analyses of clinical trials have shown a trend that patients with prior exposure to the anti-EGFR monoclonal antibody cetuximab tend to have worse outcomes when receiving subsequent ICI treatment. Here, we validated these findings in two clinical cohorts and investigated the underlying mechanisms. We confirmed that HNSCC patients with higher density of cetuximab exposure had a greater incidence of subsequent ICI resistance. In HNSCC cells continuously exposed to cetuximab, a chronic inflammatory gene expression signature, including the IFN-γ response, was strongly enriched early but gradually reduced over time. Intriguingly, cetuximab-resistant cells displayed a blunted response to IFN-γ, and STAT1 protein degradation occurred through persistent Tyr701 phosphorylation. Furthermore, STAT1 acetylation at Lys637 decreased the amount of dimeric Tyr701 phosphorylation and attenuated STAT1 transcriptional activity. Potential upstream signals for STAT1 modification under chronic cetuximab exposure include TNF-α and IFN-β. Analysis of pre-ICB samples from HNSCC patients revealed that Lys637 acetylation significantly correlates with a reduced response and worse outcomes in patients receiving ICI. This finding suggests that chronic exposure to and resistance against anti-EGFR antibodies elicit a chronic inflammatory environment, which subsequently blunts the IFN-γ response of tumor cells, thereby hampering ICI efficacy. This study highlights the potential mechanisms by which chronic use of targeted therapies results in poor ICI response and demonstrates a potential marker for guiding ICI treatment.

Title of Project: Cancer Initiation and Progression of Ovarian High-grade Serous Carcinoma Originating from the Oviduct: Role of Ovulation

Project No.: NHRI-EX113-11216BI

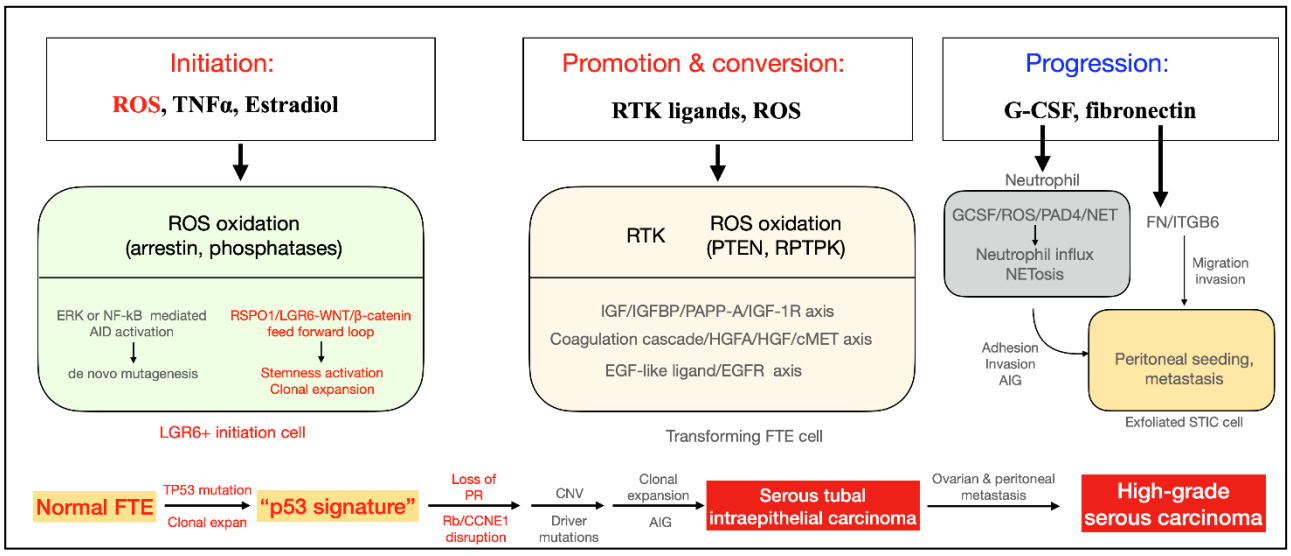
P.I. Name: TangYuan Chu/朱堂元

Key Professional Personnel: Hsuan-Shun Huang/黃玄舜, Nivethitha SK Ilavenil, Kanchana Subramani

Affiliation/Institution: Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation

Entire Project Period: From 2023 to 2025 (Total: 3 years)

In 21st-century oncology, a surprising revelation has emerged: epithelial ovarian cancers, especially high-grade serous carcinomas, predominantly originate from the fallopian tube epithelium rather than the ovary itself. This discovery challenges conventional beliefs and prompts a deeper exploration into the cell-of-origin and mechanisms driving malignant transformation within the fallopian tube. Building upon our prior findings which highlighted the role of ovulation in instigating de novo mutagenesis of the TP53 gene and fostering the malignant conversion of p53 and Rb-primed FTE cells through the release of mutagens and growth factors, this research endeavor seeks to establish a cohesive link between these pivotal stages of carcinogenesis facilitated by the process of ovulation. Our hypothesis posits that the LGR6/RSPO1 stemness niche may undergo upregulation in response to ovulatory cues. Cells expressing RSPO1/LGR6 are presumed to serve as the target for cancer initiation with TP53 mutation, and subsequently progressing towards malignant transformation. In Chapter One of this study, we have unveiled that ovulation releases reactive oxygen species (ROS) towards the exposed FTE, thereby initiating a stemness niche conducive to oncogenesis through the NOX/β-Arresting/LGR6/β-Catenin axis. In the forthcoming Chapter Two, our objective is to substantiate this proposed carcinogenic cascade by employing a triple transgenic mouse model engineered to harbor Trp53-R172H, overexpressed Ccne1, and Pgr-KO in LGR6-expressing cells. Through the sequential induction of these transgenes, we anticipate the manifestation of progressive oviduct pathology characterized by the "p53 signature," serous tubal intraepithelial carcinoma (STIC), and ultimately, HGSC.



Title of Project: Decipher the Spatiotemporal Regulation of Directional Cell Migration: from Biology to Clinical Therapies
Project No.: NHRI-EX113-11217BI
P.I. Name: Sen-Yung Hsieh/謝森永
Key Professional Personnel: Sen-Yung Hsieh/謝森永
Affiliation/Institution: Chang Gung Medical Foundation
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Ubiquitylation-degradation and Sequestration Coordinate CDC42 Turnover to Ensure Membrane Dynamics and Homeostasis during Cell Migration

F-actin cytoskeleton remodeling is essential for cell migration, organ development, and immune responses. CDC42, a factor orchestrating F-actin remodeling for membrane dynamics, switches between its inactive GDP- and active GTP-bound forms. However, the biological and clinical significance of the mechanisms regulating CDC42 protein turnover remains unclear. Here we show that KLHL23-mediated CDC42·GTP polyubiquitylation for degradation and RhoGDI-mediated CDC42·GDP sequestration away from the plasma membrane co-inactivate CDC42 in a spatiotemporal context, ensuring membrane dynamics and homeostasis during migration. Via a functional genomic screen, we identified KLHL23 as a suppressor of tumor invasion. Decreased KLHL23 level was associated with tumor metastasis and poor clinical outcomes in patients with liver and pancreas cancers. KLHL23 functions as the E3 ligase responsible for CDC42 polyubiquitylation and degradation. KLHL23 shares with RhoGDI the switch II region of CDC42 for binding, enhancing selective targeting of CDC42·GTP and CDC42·GDP, respectively. KLHL23 depletion in cells leads to F-actin and membrane over-protrusion, as well as epithelial-mesenchymal transition and tumor metastasis. Fluorescence resonance energy transfer assays reveal that the KLHL23—CDC42·GTP interaction plays a primary role in quenching CDC42 activity during membrane protrusion-retraction, while the RhoGDI—CDC42·GDP interaction occurs lately. Our results demonstrate the spatiotemporal interplay between RhoGDI and KLHL23 in regulating CDC42 turnover for membrane dynamics. As KLHL23/RhoGDI—CDC42 axis dysregulation causes tumor metastasis, our findings open avenues for exploring novel therapeutics.

Title of Project: Decoding and Modulating the Immune Synaptic Interactome for Gamma Delta T Immunotherapy in Cancer

Project No.: NHRI-EX113-11218BI
P.I. Name: Hsing-Chen Tsai/蔡幸真
Key Professional Personnel: Chong-Jen Yu/余忠仁, Tai-Chung Huang/黃泰中, Shih-Yu Chen/陳世淯, Ying-Ta Wu/吳盈達
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Adoptive cell therapy (ACT) has emerged as a promising strategy in cancer treatment. ACT involves taking immune cells from the patient, expanding them ex vivo, and infusing them back into the patient to combat cancer. Among the various killer cell types in clinical use or under active development for ACT, gamma delta (γδ) T cells are unique as they exert both innate and adaptive immune responses, such as targeting cancer cells without the need of prior activation. Moreover, their capacity to recognize cancer cells in an MHC-unrestrictive manner allows for both autologous and allogeneic therapies. Previously, our research has shown that pretreatment of cancer cells with DNA methyltransferase inhibitors can enhance γδ T-mediated cancer killing by modulating the expression of immune synaptic molecules and facilitating immune synapse formation. These findings indicate that the interactome within immune synapses between cancer and γδ T cells may dictate the therapeutic efficacy of γδ T-based cellular immunotherapy. Therefore, we aimed to characterize immune synaptic molecules and investigate how they may determine the sensitivity of lung cancer cells to γδ T-mediated killing. First, we categorized a panel of human lung cancer cells into γδ T-sensitive and γδ T-resistant cell lines using *in vitro* cytotoxicity assays. We demonstrated that the sensitive cell lines tend to form more immune synapses than the resistant ones. Furthermore, we utilized spatial optoproteomics technology (Microscoop™) to profile interacting proteins within the immune synapses. We successfully established an AI-based image recognition protocol that can simultaneously locate and photo-label immune synapses in varying shapes with an accuracy above 85%. Mass spectrometric analysis of the photo-labeled proteins revealed 688 candidate immune synaptic proteins, including canonical surface immune-related receptors/ligands (i.e., LFA-1, ICAM-1, ZAP-70, and components of γδ TCR), among other membrane proteins. Notably, we uncovered abundant primary cilia, mitochondrial, and cytoskeletal proteins, indicating their previously unrecognized significance related to the functions of immune synapses. We validated several selected candidate proteins and demonstrated their presence at or near immune synapses using immunofluorescence confocal microscopy. We will continue to investigate the differences in immune synaptic components between γδ T-sensitive and γδ T-resistant cell lines, aiming to decipher their molecular mechanisms and functional characteristics. Overall, our data demonstrated the feasibility of spatial optoproteomics technology and identified many candidate immune synaptic proteins related to γδ T killing. We believe our findings will shed light on the immune synaptic interactomes that confer sensitivity to γδ T-mediated immunotherapy, facilitating the development of γδ T-based adoptive cell therapy for treating lung cancer patients.

Title of Project: Novel Approaches for Disrupting KRAS Feedforward Loops in PDAC Treatment
Project No.: NHRI-EX113-11219BI
P.I. Name: Yuh-Pyng Sher/佘玉萍
Key Professional Personnel: Shih-Jen Liu/劉士任, Wei-Chung Cheng/鄭維中, Chun-Chieh Yeh/葉俊杰
Affiliation/Institution: China Medical University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Disrupting KRAS signals has been a challenge in pancreatic cancer therapy for over 30 years. Given that KRAS mutation diversity and the co-existence of multiple KRAS mutants in the pancreatic tumor, it is unfeasible to target a single mutant type for treatment. Thus, we provide a practical approach to triggering oncogenic KRAS protein degradation in KRAS-driven cancers. We reveal that a disintegrin and metalloproteinase domain 9 (ADAM9) contributes a feed-forward effect to enhance KRAS activity positively. This unique ADAM9-KRAS loop distinguishes cancer and normal cells' effects and becomes a targetable pathway when designing treatment strategies. Notably, the ADAM9 suppression-enhanced KRAS degradation is a universal phenomenon in pancreatic cancer harboring wild-type or mutant KRAS. We describe endogenous plasminogen activator inhibitor 1 (PAI-1) as a novel selective autophagy receptor that eliminates KRAS proteins under stress. The up-regulated PAI-1 directly interacts with KRAS and LC3 to induce the lysosomal degradation of KRAS under ADAM9-depleted conditions. Notably, developed ADAM9 inhibitors may function as pan-KRAS inhibitors across different KRAS mutants and could thus have a significant impact on pancreatic cancer treatment.

Title of Project: To Investigate the Role of NUDT16L1 in Ferroptosis Insensitivity during Colon Cancer Progression

Project No.: NHRI-EX113-11220BI

P.I. Name: Shih-Chieh Lin/林世杰

Key Professional Personnel: Yi-Syuan Lin/林逸宣, Chia-Jung Li/李佳榮, Bo-Wen Lin/林博文, Shang-Rung Wu/吳尚蓉

Affiliation/Institution: National Cheng Kung University

Entire Project Period: From 2023 to 2025 (Total: 3 years)

Recent studies have shown the potential of ferroptosis inducers in treating various cancer types. However, the lack of a systematic analysis of ferroptosis sensitivity in different cancer types and the unidentified critical regulator determining ferroptosis sensitivity during cancer progression has limited its clinical usage. Here, colorectal cancer is identified as one of the ferroptosis-insensitive cancer types by analyzing and NUDT16L1 is a novel ferroptosis repressor that contributes to this insensitivity. Notably, NUDT16L1 localizes to the mitochondria to maintain its proper function, including preventing mitochondrial DNA leakage by repression of mitochondrial permeability transition pore (mPTP) activity and secreting defective mitochondria after ferroptosis inducer treatment in colon cancer cells. Furthermore, NUDT16L1 represses CGAS-STING-STAT3 signaling pathway to promote M2 macrophage polarization by increases of LCN2 and IGFBP2 secretions in colon cancer cells. Mechanistically, NUDT16L1 enhances the stability of transcripts associated with ferroptosis inhibition by removing their noncanonical RNA caps. Our conditional knock-in and knockout of Nudt16l1 transgenic mouse models of colon cancer demonstrate the critical role of NUDT16L1 in promoting tumor growth. Clinical analyses have revealed that NUDT16L1 is specifically overexpressed in the cytoplasm of epithelial cells in colorectal cancer. The phosphorylation of NUDT16L1 is a potential underlying mechanism responsible for its cytosolic distribution and the insensitivity to ferroptosis in colon cancer cells. Finally, a specific NUDT16L1 inhibitor demonstrated its therapeutic potential *in vitro* and *in vivo*. Our results provide new insights into the crucial role of NUDT16L1 in promoting tumor growth and ferroptosis insensitivity in colorectal cancer and its potential as a therapeutic target. These findings offer a promising avenue for future colorectal cancer treatment research and clinical application.

Title of Project: The Role of Cancer-associated Myelopoiesis in Tumor Progression of Bone Metastatic Prostate Cancer and Potential Interventions

Project No.: NHRI-EX113-11221BI
P.I. Name: Chen Hui-Ming/陳繪名
Key Professional Personnel: Pei-Wen Hsiao/蕭培文
Affiliation/Institution: Academia Sinica
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Bone metastasis associated with advanced stage prostate cancer (PCa) develops skeletal-related events resulting in devastating cancer-related morbidities and poor prognosis. It is important that membrane-bound sialylation and intrinsic metastatic and dormant-to-proliferating shift signalings in $RM1^{BnMet}$ cells may not only promote tumor progression but also hinder myelopoiesis.

In the present report, we observed that $RM1^{BnMet}$ tumor-bearing mice had poor survival rate and higher bone metastatic incidence than $RM1^{parental}$ tumor-bearing mice. Besides, the $RM1^{dormant}$ cells conferred higher drug resistancy to docetaxel *in vitro* and *in vivo* assay when compared to $RM1^{proliferating}$ cells. Furthermore, we performed the scRNA on bone marrow tissues from $RM1^{proliferating}$ vs $RM1^{dormant}$ tumor-bearing mice with/without docetaxel treatment. Besides, we have identified several molecules/sialoproteins from multi-omics analysis (RNAseq, proteomic and membrane glycopeptidyl analysis) of $RM1^{parental}$ vs $RM1^{BnMet}$ and $RM1^{proliferating}$ vs $RM1^{dormant}$ mouse prostate cancer cells. The potential molecules are going to be evaluated for their roles in tumor progression as well as Sialic Acid-Siglec-F axis. We also observed that the blockade of siglec-F can regulate the phenotypical changes of tumor-associated bone marrow cells. All above data is under analysis and will be further confirmed.

Understanding these sophisticated networks underpinning bone metastasis is critical to provide a nurturing niche for ameliorating the quality of cancer patients' life and exploiting potentially curative treatments when malignant cells remain controllable.

Title of Project: Study and Modulation of the Brain Tumor Microenvironment for Brain Disease Therapeutics Development

Project No.: NHRI-EX113-11302BI

P.I. Name: Kuo-Chen Wei/魏國珍

Key Professional Personnel: Hao-Li Liu/劉浩澧, Hung-Wei Yang/楊閎蔚, Bertrand Chin-Ming Tan/譚賢明, Ko-Ting Chen/陳科廷, Ya-Jui Lin/林亞銳, Chiung-Yin Huang/黃瓊瑩

Affiliation/Institution: Chang Gung Medical Foundation

Entire Project Period: From 2024 to 2026 (Total: 3 years)

Gliomas are the most common tumors in central nervous system, and glioblastoma multiforme is the most malignant type. Despite of aggressive treatment, the average survival of patients is only 14 months. Malignant glioma is composed of a highly heterogeneous tumor cell population, the tumorigenesis, invasion and progression are closely related to the interaction with its surrounding microenvironment, such as neuron, vascular cells, glial cells, immune cells and physiological factors. To further understand these interactions and its patient prognosis association, we constructed a bulk RNA deconvolution method, the quantifying proportion of cells for GBM (QPC-GBM), to estimate cell 6 compositions in GBM RNA samples. By analyzing TCGA-GBM and CGGA-GBM datasets, we found the abundance of tumor and endothelial cells were positively correlated to each other, whereas their abundances were negatively correlated to GAMs and mural cells. Furthermore, QPC-GBM were correlated to survival and may serve as novel drug targets. This study provides a new method for studying GBM TME and links GBM TME cells to patient survival that drives new thoughts to future GBM therapy. For development of novel brain therapeutics, we focused on sonodynamic therapy (SDT) for transcranial noninvasive brain tumor treatment. The sonosensitizer is the key component of SDT, choosing an appropriate sonosensitizer will help to enhance the treatment response. We used somatic and brain tumor model animals to evaluate the effect of two sonosensitizers, 5-ALA and fluorescein. The data suggest that the tumor inhibitory effect of both sonosensitizers is equivalent in subcutaneous tumors. In early-stage brain tumors with relatively intact blood-brain barriers, 5-ALA penetrates well and promotes tumor inhibitory effect by SDT, while fluorescein fails to accumulate in tumor area and, no therapeutic effect was observed. In conclusion, both fluorescein and 5-ALA are safe and effective SDT sonosensitizers, and the tumor microenvironment and pathologic type should be considered in the selection of adequate sonosensitizers. In this project, we aim to explore the microenvironment of brain tumors to find therapeutic strategies and develop novel non-invasive treatments that can improve the efficiency of malignant brain tumor therapies, benefiting patients.

Title of Project: Molecular Mechanisms of Arl4A/D Small GTPases Signaling in Cancer Development

Project No.: NHRI-EX113-11306BI
P.I. Name: Fang-Jen Lee/李芳仁
Key Professional Personnel: Chia-Tang Chen/陳迦燈, Ming-Chieh Lin/林明潔
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2024 to 2026 (Total: 3 years)

Exosomes are a subtype of extracellular vesicles whose release is associated with the fusion of multivesicular bodies (MVBs) with the plasma membrane in cells. Previous studies have shown that the syndecan-syntenin-ALIX axis plays an important role in regulating exosome biogenesis, in a partially ESCRT-dependent manner. ADP-ribosylation factor (Arf)-like protein 4A (Arl4A) acts at the plasma membrane to mediate cytoskeletal remodeling and cell migration. Recently we have shown that endosomal GDP-bound Arl4A attenuates EGFR degradation by binding to the ESCRTII-complex. Using yeast two-hybrid, we identified Arl4A-T51N bound to syntenin. We hypothesize that the small GTPase Arl4A may play a role in syndecan-syntenin-ALIX axis-dependent exosome biogenesis. We first showed that Arl4A interacts with syntenin both *in vitro* and *in vivo*. We also showed that knocking down Arl4A reduces the amount of exosome secretion in the lung adenocarcinoma cell line PE089. By immunofluorescence assays, we found that Arl4A and syntenin are colocalized at $CD63^+$ late endosome compartments in cells. Further interaction analysis revealed that both Arl4A and the exosome-regulatory protein CD63 bind to syntenin via the PDZ1 domain and the C terminus of syntenin and that the three proteins have the potential to form a complex. We have also shown that Arl4A inhibits the colocalization of syntenin with the lysosomal marker Lamp1, which may explain the mechanism of how Arl4A regulates exosome biogenesis via the syndecan-syntenin-ALIX pathway. Although further studies are required to decipher the mechanism in detail, our study reveals a novel role of Arl4A as a potential regulator in extracellular vesicle biogenesis via interaction with syntenin.

Title of Project: Develop Next Generation Dual Specific Modulators Targeting Receptor Tyrosine Kinases

Project No.: NHRI-EX113-11308BC
P.I. Name: Po-Han Chen/陳伯翰
Key Professional Personnel: Po-Han Chen/陳伯翰
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2024 to 2027 (Total: 4 years)

Receptor tyrosine kinase (RTK) signaling is essential for proper cell proliferation. Dysregulation of RTK signaling is often found in oncogenesis or other human diseases. Prominent examples include dysregulated epidermal growth factor receptors (EGFR) in lung cancer, which is one of the leading cancers in the Taiwanese population. RTK inhibitors have thus been developed and widely applied for targeted therapies. However, acquired resistance and potential adverse effects of traditional drugs have prompted the drug industry to discover novel therapeutic tools.

The discovery of bifunctional molecules such as proteolysis-targeting chimeras (PROTACs) and lysosome-targeting chimeras (LYTACs) have provided novel avenues for targeted protein regulation. Compared with RTK small molecule inhibitors or antibodies, RTK-PROTAC or LYTAC degrades RTK and exhibits a different pharmacological mode of action in regulating RTK signaling. However, targeted RTK degradation may not provide optimal therapeutic benefits, given the role of RTKs, as the central hub to accept extracellular signals, integrate, and transduce the signal to downstream regulators.

Here, we provide a new concept of targeted RTK regulation using bifunctional molecules. We propose RTK phosphorylation targeting chimeras (PhosTACs), which recruits a phosphatase to the RTK-of-interest and to re-program RTK activities. Specifically, we used chemical biology approaches to generate the proof-of-concept EGFR PhosTACs in the engineered cervical and lung cancer cell lines. We combined EGFR small molecule inhibitors gefitinib or afatinib with the tyrosine phosphatase-FKBP12(F36V) fusion protein recruiting elements to generate EGFR PhosTACs- GePhos1 or AfaPhos1, respectively. The dual mode of action, derived from the RTK inhibitors and phosphatases, of EGFR PhosTACs repressed EGFR activities comparable to the RTK inhibitors. Using phosphoproteomic analysis, we found EGFR PhosTACs demonstrated distinct effects in regulating downstream phosphorylation signaling compared with gefitinib alone. Besides the canonical EGFR targets, PhosTAC treatment significantly downregulates phosphorylation events associated with RNA processing and chromatin organization. EGFR PhosTACs also reduce cell viability and increase apoptosis in the engineered cell lines.

In sum, the EGFR PhosTACs showcased the first example of heterobifunctional small molecules harnessing a tyrosine phosphatase for targeted protein dephosphorylation. The unique dual mode of action embedded within the EGFR PhosTAC exhibits a new avenue for future drug discovery targeting RTK.

Title of Project: Deciphering the Mechanistic Link between PCNA Tyrosine Phosphorylation and Anti-tumor Immunity: Implications for Immuno-oncology Therapy

Project No.: NHRI-EX113-11317BI

P.I. Name: Shao-Chun Wang/王紹椿

Key Professional Personnel: Chuan-Chun Lee/李權峻, Wan-Rong Wu/鄔宛蓉, Yi-Chun Shen/沈宜君, Feng-Chi Chung/鍾鳳祁, Yuan-Liang Wang/王元良, You-Zhe Lin/林佑哲, Chih-Hao Lu/陸志豪, Wei-Chung Cheng/鄭維中, Han Chang/張涵, Liang-Chih Liu/劉良智, Ji-An Liang/梁基安, Chang-Fang Chiu/邱昌芳, Mien-Chie Hung/洪明奇

Affiliation/Institution: China Medical University

Entire Project Period: From 2024 to 2026 (Total: 3 years)

Cancer cells are continually exposed to intrinsic and extrinsic proliferative stresses that can send the genome stability awry. The aberrant DNA metabolism, evolving through deregulated replicative growth in cancer cells, is increasingly recognized as a major mechanism bridging the crosstalk between the immune microenvironment and tumor tissue, thereby either promoting or suppressing tumor progression. However, the triggering and regulation of these mechanisms remain largely unknown. Proliferating cell nuclear antigen (PCNA) and its homologs are the evolutionarily conserved central components of the DNA replication machinery. In eukaryotic cells, PCNA forms homotrimeric rings encircling the DNA double helix to coordinate the complex processes of DNA replication and damage repair, directed at least in part by differential post-translational modifications of the PCNA protein. In cancer cells, growth signal-stimulated phosphorylation at tyrosine 211 of PCNA (pY211-PCNA) is known to play an important function for active proliferation. Here, we demonstrate that inhibition of pY211 disrupts the processivity of replication forks. This disruption triggers single-stranded DNA (ssDNA) production catalyzed by the MRE11 nuclease, activating the cGAS-STING axis to launch an anti-tumor immune response by natural killer (NK) cells. We further show that the pY211 regulates site-specific post-translational modifications of MRE11, and loss of pY211 promotes the endonuclease mode of MRE11. This causes ssDNA generation in the cytosol, subsequently inducing type I interferon-activated cytotoxicity by NK cells and fostering an anti-tumor immunity that abrogates distant metastasis. Mechanistically, the pY211 determines the recruitment of the writer or eraser of the modification on MRE11, thus tuning the nucleolytic modes of MRE11 and, consequently, ssDNA generation. Therapeutic measures promoting MRE11-dependent ssDNA enhance the response of triple-negative breast cancer to cytotoxic killing mediated by endogenous or allogenic NK cells in mice. In breast cancer patients, the level of cytosolic ssDNA is inversely correlated with the expression of the eraser and pY211-PCNA, which is associated with poor overall survival in cancer patients. Our studies shed new light on the shaping of the tumor immune microenvironment and demonstrate the potential to develop therapeutic approaches that proactively leverage genomic instability and immune activation to target malignant tumors.

Title : DUSP22 Inhibits Lung Tumorigenesis by Suppression of EGFR/c-Met Signaling

P.I. Name : Wen-Jye Lin/林文傑
Presenter : Yu-Ting Liao, Wen-Jye Lin
Institute/Center : National Health Research Institutes

DUSP22, an atypical dual-specificity phosphatase enzyme, plays a significant role in regulating multiple kinase signaling pathways by dephosphorylation. Our study reveals that decreased DUSP22 expression is associated with shorter disease-free survival, advanced TNM (tumor, lymph nodes, and metastasis), cancer stage, and higher tumor grade in lung adenocarcinoma (LUAD) patients. Exogenous DUSP22 expressing reduces the colony-forming capacity of lung cancer cells and inhibits xenograft tumor growth primarily by targeting EGFR and suppressing its activity through dephosphorylation. Knockdown of DUSP22 using shRNA enhances EGFR dependency in HCC827 lung cancer cells and increases sensitivity to gefitinib, an EGFR inhibitor. Consistently, genetic deletion of DUSP22 enhances EGFRdel (exon 19 deletion)-driven lung tumorigenesis and elevates EGFR activity. Pharmacological inhibition of DUSP22 activates EGFR, pERK1/2, and upregulates downstream PD-L1 expression. Additionally, lentiviral deletion of DUSP22 by shRNA enhances lung cancer cell migration through EGFR/c-Met and PD-L1-dependent pathways. Gefitinib, an EGFR inhibitor, mechanistically suppresses migration induced by DUSP22 deletion and inhibits c-Met activity. Furthermore, cabozantinib, a c-Met inhibitor, reduces migration and attenuates EGFR activation caused by DUSP22 deletion. Collectively, our findings support the hypothesis that loss of DUSP22 function in lung cancer cells confers a survival advantage by augmenting EGFR signaling, leading to increased activation of downstream c-Met, ERKs, and PD-L1 axis, ultimately contributing to the progression of advanced lung cancer.

Title of Project: Design and Optimization of Advanced Xeno-free Smart Microcarrier Automation MSC Enrichment System

Project No.: NHRI-EX113-11103EI
P.I. Name: Dar-Bin Shieh/謝達斌
Key Professional Personnel: Pei-Wen Wang/王佩文
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

The global stem cell market, valued at $132.66 billion in 2022, is projected to grow at a compound annual growth rate (CAGR) of 9.74% from 2023 to 2030. Regenerative medicine and drug development are the main pillars of the stem cell industry. Taiwan's demand for automated stem cell isolation and cultivation led to a multidisciplinary collaboration integrating molecular cell biology, biochemical engineering, materials science, biotechnology, nanotechnology, and clinical medicine. This project developed a closed-loop system for automated MSC cultivation using temperature-sensitive polymers and zero-valent iron nanoparticles (NP-Z). The serum-free, xenogeneic-free medium, in combination with NP-Z, promotes MSC growth and maintains their stemness. Low-dose NP-Z (0.2-1 μg/mL) enhances MSC growth without cytotoxicity and improves cell crawling ability, delays aging, and supports differentiation into fat cells, osteoblasts, and chondrocytes. We explored compounds inducing ferroptosis like NP-Z, finding only low-concentration Erastin (1-5 μM) mildly promotes MSC growth while retaining stem cell markers. Transitioning from 2D to 3D culture systems, we collaborated with Professor Hsieh-Chih Tsai to develop temperature-sensitive polymer coatings. We combined NP-Z nanoparticles with hydrophilic positively charged allylamine (ALA) using the GLA microsphere carrier to synthesize superparamagnetic microcarriers (GLA@ZVI@ALA). By testing different ratios of NP-Z and ALA synthesis encapsulation, we determined that the optimal weight ratio of GLA:ZVI = 15:1:5 maintains magnetism conducive to MSC adsorption and growth, and achieves smooth cell detachment after cooling to 4°C. These polymers allow MSC adhesion at 37°C and natural detachment at 4°C, optimizing MSC growth conditions. A temperature control module using PI-embedded carbon film heaters ensures a stable culture medium temperature of 37°C. A gas concentration controller and heating chamber simulate real cell culture conditions, with calibrated gas sensors ensuring accurate measurements. Liquid volume control achieved precise liquid transfer, while an optical system enabled effective cell survival quality control. The multifunctional automated cultivation system ensures a stable environment for consistent cell cultivation, with future plans focusing on further optimization, expanded research, and practical clinical trials.

Title of Project: Adaptable Immunotherapeutic Spray Enabled Fenton Nanocatalytic Cancer Therapy for Suppression of Postoperative Malignant Glioma Recurrence

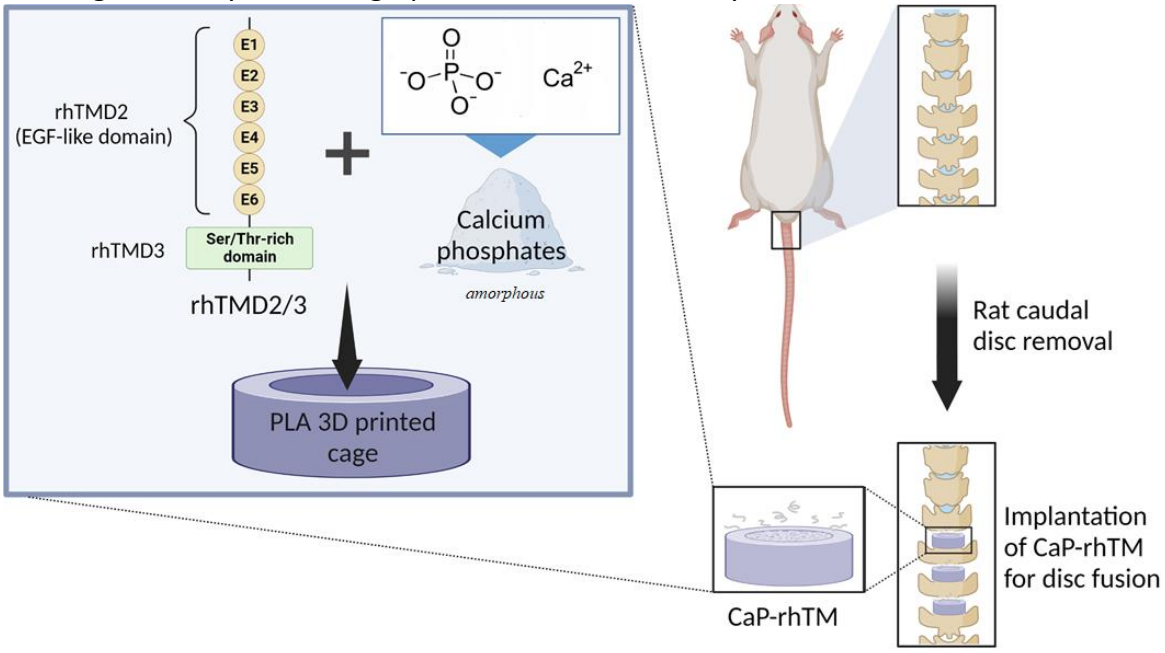
Project No.: NHRI-EX113-11111EI
P.I. Name: Shang-Hsiu Hu/胡尚秀
NHRI Researcher: Lun-De Liao/廖倫德
Key Professional Personnel: Shang-Hsiu Hu/胡尚秀
Affiliation/Institution: National Tsing Hua University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Endogenous signals, including nitric oxide (NO) and electrons, play pivotal roles in orchestrating diverse physiological processes involved in cell fate regulation, as well as in the modulation of vascular and neuronal systems. However, leveraging these signals in clinical contexts faces challenges due to the short-lived nature of NO and the limited means to precisely control the spatiotemporal release of gases and electrical impulses. In this study, we propose a novel approach dubbed the "magnetoelectric massager" strategy, which harnesses alternating magnetic fields (AMF) to trigger on-demand release of NO and induce electrical stimulation for the restoration of brain function following traumatic brain injury. Upon exposure to AMF irradiation, the conductive MoCx-Cu generates eddy currents that facilitate the release of NO from GSNO via electrical stimulation. This mechanism significantly enhances the differentiation and growth of neural stem cell (NSC) synapses. When applied *in vivo* to models of traumatic brain injury, this combined approach not only promotes the inhibition of inflammation and angiogenesis but also facilitates neuronal interrogation, thereby offering a comprehensive strategy for mitigating the consequences of brain trauma.

Title of Project: CAP-resin-rhTM as Sustained Release Bone Cement for the Stimulation of Spinal Fusion in Intervertebral Disc of Rat Tail Model
Project No.: NHRI-EX113-11113EC
P.I. Name: Yan-Jye Shyong/熊彥傑
Key Professional Personnel: Cheng-Li Lin/林政立
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Calcium Phosphate Complex of Recombinant Human Thrombomodulin Promote Bone Formation in Interbody Fusion

Interbody fusion is an orthopedic surgical procedure to connect two adjacent vertebrae in patients suffering from spinal disc disease. The combination of synthetic bone grafts with protein-based drugs is an intriguing approach to stimulate interbody bone growth, specifically in patients exhibiting restricted bone progression. Recombinant human thrombomodulin (rhTM), a novel protein drug characterized by its superior stability and potency, shows promise in enhancing bone formation. A composite bone graft, termed CaP-rhTM, has been synthesized, combining calcium phosphate (CaP) microparticles as a delivery vehicle for rhTM to facilitate interbody fusion. *In vitro* studies have demonstrated that rhTM significantly promotes the proliferation and maturation of preosteoblasts at nanogram dosage, while exerting minimal impact on osteosarcoma cell growth. The expression levels of mature osteoblast markers, including osteocalcin, osteopontin, alkaline phosphatase, and calcium deposition were also enhanced by rhTM. In rat caudal disc model of interbody fusion, CaP-rhTM with 800 ng of drug doaeage was implanted along with a polylactic acid (PLA) cage, to ensure structural stability within the intervertebral space. Microcomputed tomography analyses revealed that from 8 to 24 weeks, CaP-rhTM substantially improves both bone volume and trabecular architecture, in addition to the textural integrity of bony endplate surfaces. Histological examination confirmed the formation of a continuous bone bridge connecting adjacent vertebrae. Furthermore, biomechanical assessment via three-point bending tests indicated an improved bone quality of the fused disc. This study has demostrated that rhTM exhibits considerable potential in promoting osteogenesis. The use of CaP-rhTM has also shown significant improvements in promoting interbody fusion. A graphic abstract of this study is demonstrated below.



Title of Project: Development of Therapeutic Nanoconnectors for Keratoconic Corneal Tissue Repair
Project No.: NHRI-EX113-11128EI
P.I. Name: Jui-Yang Lai/賴瑞陽
Key Professional Personnel: Chia-Jung Yang/楊佳蓉, Yun-Han Su/蘇筠涵
Affiliation/Institution: Chang Gung University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

Purpose: Considering the low bioavailability of eye drops and the multiple symptoms associated with keratoconus (such as oxidative stress, inflammatory response, collagen degradation, keratocyte transdifferentiation, and tissue weakening), the development of novel nanotherapeutics has the potential to achieve comprehensive treatments and tackle the problem at its roots. Here, we investigate systematically the role of grafting amount and molecular weight of biopolymers in the properties of multifunctional bioconjugates (i.e., nanoconnector (NC)) composed of chitosan (CS), gallic acid (GA), hyaluronan (HA), and collagen-mimetic peptides for keratoconic tissue repair. **Methods**: A series of biomaterial samples were prepared via carbodiimide chemistry. Following chemical characterizations, the bioactive supramolecular assemblies were evaluated by determinations of their antioxidant/anti-inflammatory/anti-matrix metalloproteinase (MMP) activities and capabilities of stimulating matrix biosynthesis and inhibiting keratocyte transdifferentiation. Then, the CSGAHA was functionalized with multi-arm PEGylated collagen-mimetic peptides (PG) of three varying amounts (0.1, 0.5, and 1 mmol) to obtain the NC(L), NC(M), and NC(H), respectively. The multifunctional bioconjugates were further assessed for their therapeutic performance in a rabbit model of keratoconus. **Results**: *In vitro* assays indicated that the modification of CS with GA can contribute to the antioxidant/anti-inflammatory/anti-MMP activities. Our results also showed that CSGA samples exhibited varying activity levels, which are directly correlated with grafting amounts of GA. Due to the presence of HA chains in the bioactive supramolecular assemblies, the cultured keratocytes exposed to the CSGAHA samples can synthesize matrix components (i.e., collagen and glycosaminoglycan) and maintain phenotypic expression. In particular, the CSGAHA1511 cultures showed the highest levels of matrix deposition and were the most effective in preventing cell transdifferentiation among all groups studied. Given that corneal thinning and weakening is closely linked to disease progression of keratoconus, we further examined the ability of the multifunctional bioconjugates to strengthen the tissue architecture. The results showed that NC composed of varying amounts of PG exhibited different efficiencies in hydrogen bonding-mediated collagen cross-linking. Specifically, NC(H) demonstrated the most potent effects in reducing corneal bulging and preserving tissue transparency/strengthen. In a rabbit model of keratoconus, the NC-based eye drops displayed exceptional antioxidant, anti-inflammatory, and anti-apoptotic properties, as well as the abilities for phenotypic maintenance. Notably, the results of immunohistochemistry confirmed complete suppression of MMPs in keratoconic tissues, thereby indicating the eradication of pathogenic factors. More importantly, the reinforcement of tissue matrix led to an ordered parallel arrangement of collagen fibers, which may facilitate the restoration of corneal thickness, curvature, transparency, and mechanical stability to a level similar to that of healthy eye. **Conclusion**: In summary, the results of this project suggest multifaceted bioactivities and potential application of multifunctional bioconjugates. Specifically, increasing the molecular weight of HA on NC facilitates the matrix biosynthesis and phenotypic maintenance, which supports the repair of keratoconic corneal tissue. Our work also reflects the significance of grafting amounts of PG, which can reduce corneal distortion and preserve tissue transparency. Furthermore, within 5 days of the treatment, the most comparable halt in keratoconus progression was observed in rabbit eyes treated with the NC(H) sample. The therapeutic nanoconnector holds high promise in corneal tissue engineering application.

Title of Project: Impact of Long-term Sleep Deprivation on Gut-Brain Axis in Medical Personnel: Cross-specie Intervention on Neuroimaging and Gut Microbiota Analyses

Project No.: NHRI-EX113-11129EI

P.I. Name: Changwei Wesley Wu/吳昌衛

Key Professional Personnel: Lun-De Liao/廖倫德, Changwei W. Wu/吳昌衛, Yu-Tang Tung/童鈺棠, Yi-Ping Chao/趙一平, Chih-Mao Huang/黃植懋

Affiliation/Institution: Taipei Medical University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

We hypothesized that the systemic interactions of brain-gut-microbiota axis (BGMA) can reflect the cognitive decline following a long-term sleep deficiency, and performed multi-modal measures to assess the neurophysiological alterations following chronic sleep deprivations (CSD) in animal model and human participants. We split the project into two protocols with the following details.

1. **Antioxidant effect on mice model with CSD:** Using the developed mice models of CSD (28 consecutive days with 10-hour SD per day) in National Chung Hsing University, we separated the mice into 4 groups (n=6 each): normal sleep (NS), chronic sleep deprivation (CSD), chronic sleep deprivation with additional 1-week sleep recovery (CSDR), and chronic sleep deprivation with calcitriol (CSDVD). After the group-specific manipulations, we conducted multiple behavioral tests, collected fecal samples for the analyses of gut microbiota, short-chain fatty acid (SCFA). In behavior, the open field test (OFT) revealed that both CSDR and CSDVD could increase the locomotor activity following CSD. In microbiota composition, the principal coordinate analysis (PCoA) based on weighted-unifrac distances unclosed that the beta diversity of the calcitriol-supplied group (CSDVD) closely matches the microbiome pair distances of the NS group, distant away from the CSD and CSDR groups. The *Firmicutes* to *Bacteroidetes* ratio (F/B ratio), an index reflecting the symbiotic microbiota balance, also recovered after calcitriol supplementation, indicating a reduction in gut microbiota dysbiosis following CSD. In SCFA, the Acetic acid did not change across groups; the CSD and CSDR groups had lower Propionic acid compared to the NS, which recovered back to normal in the CSDVD only; the Butanoic acid was only lowered in the CSD group, returned to normal in both CSDR and CSDVD.
2. **Alteration of BGMA and neurocognitive function after night shifts in human:** We recruited 10 human participants who are currently medical personnel worked with night shifts, collecting their fMRI (brain imaging), fecal samples (gut microbiota and short-chain fatty acid, SCFA), and neuropsychological assessments (cognition) at 3 timings – baseline, immediately after night shift, and a 4-day post-rest with day shift. Results indicated that 5-day night shifts lead to a partial sleep deprivation and decreased the processing speed of executive control functions. Night-shift work also altered brain functional connectivity (FC) within the executive control network (increased FC after night shift) and the default-mode network (DMN, decreased FC after night shift), which did not recover during the post-rest. Similarly, gut microbiota did no show significant timing differences in the alpha/beta diversity, SCFA levels, neither did the microbiota concentration at the phylum level. Nonetheless, the gut-brain interactions after night shifts showed the negative correlation between the DMN and the abundance of *Proteobacteria* and *Bacteroidota* at the family level. These findings implied that (1) the circadian desynchrony due to night shifts showed profound impacts on the brain functionality, but not the gut microbiota, and thus deteriorates the cognitive performances in medical professions, and (2) the 4-day post-rest with circadian realignment might be insufficient to undo the night-shift-induced deteriorations of cognitive performances and brain functionality.

Title of Project: Development of a Wearable Ultrasound Device to Optimize the Treatment of Rotator Cuff Tear by Characterizing Dynamic Properties of the Shoulder Tissues

Project No.: NHRI-EX113-11130EI
P.I. Name: Chih-Chung Huang/黃執中
Key Professional Personnel: Fong-Chin Su/蘇芳慶, Wei-Ren Su/蘇維仁, Li-Chieh Kuo/郭立杰
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2022 to 2024 (Total: 3 years)

The shoulder is the most mobile joint in the human body, thus requiring intricate coordination of adjacent muscles. Any imbalance of force couples makes an individual prone to rotator cuff disease in the long run. Approximately 30% of people above 60 years old suffered from rotator cuff tear. Typical symptoms include shoulder pain and difficulty raising the arm, thus reducing work efficiency and compromising the quality of life. Partial-thickness rotator cuff tears are generally managed with analgesics and rehabilitation, while full-thickness rotator cuff tears were preferably managed with surgery. Unfortunately, arthroscopic repairs offer patient with massive and chronic tears little benefit. Advanced rotator cuff tear is often complicated with stiffness, atrophy, and fatty infiltration of the rotator cuff muscles and tenons, which significantly reduce mobility of the tendon during surgery. These degenerative changes not only lead to incomplete repairs, but also increase the chance of re-tear months after surgery. Ultrasound has been used widely for shoulder soft tissue imaging. It not only provides the structural information of shoulder but also blood flow conditions. Recently, considering the dilemma of treating degenerative rotator cuff tears, ultrasound elastography was introduced in shoulder examination. By computing the elastic modulus of rotator cuff tendons, surgeons could now assess the extensibility of tendons preoperatively and track the quality of healing tendon postoperatively. Besides the muscles and tendon elasticity, another important role in tendon injury is the blood supply. Unhealthy blood supply may lead the disorder of shoulder healing. Unfortunately, there is no real-time device that can be used to measure the blood flow information of shoulder except Doppler ultrasound imaging. However, most ultrasound examinations are under a static condition. Providing dynamic information from shoulder positions of shoulder during moving. Therefore, 1) we are going to develop a “wearable” ultrasound imaging device which combines with optical motion tracking system in this three-years project. It allows us to track the shoulder position and its corresponding ultrasound functional images synchronously. This wearable device will include 2) high frame rate ultrasound elastography, 3) high resolution blood flow mapping, 4) Doppler vector imaging, and 5) muscles and tendon motion tracking imaging. The development of this wearable ultrasound device and new imaging technologies can make tremendous contributions to the diagnosis and treatment of rotator cuff disease. 6) Preoperatively, the surgeons will be able to assess the elasticity as well as vascular supply in a non-invasive approach under a dynamic condition. The information will be invaluable for presurgical planning and prediction of successful rate. 7) During surgery, the blood flow conditions well be measured for assessing the operational strategy. 8) Postoperatively, the device will allow the therapists to assess the rotator cuff muscles while the patients are performing physiological movements. Also, the new device can quantify the progress of healing, thereby helping surgeons and physical therapists determine whether the intensity of rehabilitation shoulder be tuned down or escalated.

Title of Project: 3D Stem Cell Spheroid-derived ECM as an Immunomodulatory Scaffold System for Regenerative Medicine

Project No.: NHRI-EX113-11131EI

P.I. Name: Chieh-Cheng Huang/黃玠誠
Key Professional Personnel: Chieh-Cheng Huang/黃玠誠
Affiliation/Institution: National Tsing Hua University

Entire Project Period: From 2022 to 2024 (Total: 3 years)

Tissue engineering seeks to develop scaffolds that simulate the native extracellular matrix (ECM) by providing physical support and biochemical cues to regulate various cellular processes. However, current research primarily employs biomaterials as scaffolds based solely on their structural and mechanical properties, without offering the crucial environment necessary for promoting tissue regeneration. In this study, we developed a bioactive tissue-engineering scaffold system using three-dimensional (3D) decellularized ECM (dECM) derived from mesenchymal stem cell (MSC) spheroids. Our approach utilized a surfactant-based decellularization method that effectively removes cells while retaining the matrix composition and MSC secretome within the obtained 3D dECM constructs. Proteomic and cytokine array analyses revealed that 3D dECM retained a diverse array of MSC spheroid-derived matrisome proteins and secretome components, crucial for replicating the complexity of native ECM and the therapeutic capabilities of MSCs. These molecules were found to underlie the observed effects of 3D dECM on immunomodulation, proneuritogenesis, and proangiogenesis in our *in vitro* functional assays. Implantation of 3D dECM into mice with traumatic brain injury (TBI) effectively mitigated post-injury tissue damage and promoted brain repair. This was evidenced by a reduced brain lesion volume, decreased cell apoptosis, alleviated neuroinflammation, reduced glial scar formation, and increased neuroblast recruitment to the lesion site. These outcomes culminated in improved motor function recovery in the animals, highlighting the multifaceted therapeutic potential of 3D dECM for TBI. In summary, our study elucidates the transformative potential of MSC spheroid-derived bioactive 3D dECM as an implantable biomaterial for effectively promoting tissue regeneration, paving the way for its broader therapeutic application.

Title of Project: Deep Learning-enhanced Ultra-low-count tau PET Neuroimaging
Project No.: NHRI-EX113-11205EC
P.I. Name: Kevin Tze-Hsiang Chen/程子翔
Key Professional Personnel: Kevin Tze-Hsiang Chen/程子翔
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2026 (Total: 4 years)

With deep learning-based methods, we have previously demonstrated the use of U-Net-based methods that incorporate spatially correlated multimodal MR and PET information to produce high quality [^{18}F]-florbetaben amyloid PET images from scan protocols with markedly reduced injected radiotracer dose (as low as ~1% dose). However, for tau radiotracers, where the uptake is focal, has greater image noise, and the signal is weaker than that of amyloid, we have also shown in the second-generation tau tracer [^{18}F]-PI-2620 that it is important to take data bias stemming from the use of different tracers into account and to either re-train or fine-tune networks using tracer-specific data. In the first stage of this project, we have shown that these challenges related to the tau PET image warrants further investigation in other related radiotracers and dose reduction levels. Subsequently, we aimed to investigate whether a UNet-based network can enhance ultra-low-dose [^{18}F]-APN-1607 tau PET images and whether the generated images are qualitatively and quantitatively accurate.

46 participants (26 female, 2 unknown; 66.89±9.52 years), of which 6 were scanned twice, were recruited to train the ultra-low-dose tau network; the participants included individuals with a range of neurodegenerative disorders as well as healthy controls. 274±82 MBq of the tau radiotracer [^{18}F]-APN-1607 was injected. Static PET data, subdivided into 6 5-minute subframes, was acquired 90-120 minutes post-injection. The subframe corresponding to 90-95 minutes post-reconstruction was chosen to simulate the ultra-low-dose PET image (~1/6 dose); T1-weighted MR images were acquired separately prior to the PET scan. Our network is based on UNet3+ by Huang, Huimin et al., which enhances the traditional UNet architecture with full-scale skip connections. These connections integrate feature maps from different scales, capturing both fine-grained details and coarse-grained semantics. For improved performance in low-dose enhancement, we replaced the activation function of the output layer from a sigmoid function to a ReLU function. The dataset was split according to the ratio of training:validation:testing = 38:6:8 with random vertical flips, rotations, translation, and resizing for data augmentation. The network was trained with batch size 16 over 80 epochs with an initial learning rate of 1e-4, scheduled using CosineAnnealingLR and AdamW as the optimizer.

For each axial slice of the volumes, the image quality of the enhanced PET and the ultra-low-dose PET images were compared to the original full-dose image using the metrics peak signal-to-noise ratio (PSNR), structural similarity (SSIM), and mean-squared error (MSE). The values were weighted by voxel number per slice and averaged to derive a set of metrics per participant. Wilcoxon tests at the $p = 0.05$ level and Bonferroni correction for multiple comparisons were used to compare the metrics derived from different image types.

Qualitatively, the synthesized images show marked improvement in noise reduction compared to the low-dose image and resemble theirs full-dose counterparts. Quantitatively, the three metrics all improved significantly after enhancement ($p<0.05/3$). In conclusion, this work has shown that high-quality tau PET images can be generated using deep learning methods; future work for subsequent years of this project include further quantitative evaluation such as region-based standard uptake value ratio analyses and reader studies.

Title of Project: Immunofoam: an Innovation for Intracavitary Combination Therapy to Solid Tumors Using Biomaterials-assisted Immunotherapy and Sonoporation-enhanced Drug Penetration

Project No.: NHRI-EX113-11206EC
P.I. Name: Yen-Liang Liu/劉彥良
Key Professional Personnel: Ulziijargal Sukhbat, Hsin-Yi Lin/林欣儀, Chin-Yi Yeh/葉沁怡
Affiliation/Institution: China Medical University
Entire Project Period: From 2023 to 2026 (Total: 4 years)

Ovarian cancer (OC) is a formidable adversary in women's health. OC is often diagnosed at advanced stages with pervasive metastasis, necessitating innovative therapeutic paradigms. Here, we aim to develop ultrasound-assisted chemotherapy for treating OC-associated peritoneal metastasis. We have developed an ultrasound-responsive liquid foam that can serve as a drug carrier and perform sonoporation to enhance drug penetration into deep tumors. The foam formulation enables drug carriers to conform to the tissue surface and immerse the cancer cells in therapeutic agents, extending the drug contact time. Ultrasound-responsive microbubbles can further enhance drug penetration through ultrasound-triggered sonoporation. We evaluated the sonoporation using variable ultrasound intensity and duration, and the sonoporation efficacy is proportional to the ultrasound intensity and treatment duration. The cytotoxicity (IC50) of cisplatin and carboplatin to ID8 ovarian cancer cell line was improved more than 100 folds using foam with ultrasound treatment. Our preliminary results in the metastatic ovarian mouse model demonstrated that the foam-carried chemo drugs can penetrate tumors with a depth of 200 μm and achieve better progression after treatment, highlighting the potential for improved treatment outcomes. Immunofoam, ultrasound-assisted intraperitoneal chemotherapy, addresses the current limitations in peritoneal metastasis management and presents a paradigm shift by optimizing therapeutic efficacy while minimizing systemic toxicity. We envision that our approach is promising to improve patient prognosis and contribute substantially to the evolution of precision medicine in OC management. Evaluate the efficacy of ultrasound-enhanced chemotherapy in enhancing drug penetration and cytotoxicity *in vivo*.

Title of Project: Translational Investigation of Very Low Intensity Ultrasound on the Treatment of Degenerated Intervertebral Disc
Project No.: NHRI-EX113-11222EI
P.I. Name: Jaw-Lin Wang/王兆麟
Key Professional Personnel: Guan-Jen Chen/陳冠蓁
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Results of Ultrasound and Piezoelectric Stimulation on 3D Hydrogel Needle Punctured Model

[Introduction]

Degeneration of intervertebral disc (IVD) and especially that of nucleus pulposus (NP) is correlating to the over compression of IVD by mechanical loadings, in some cases our overweight bodies. NP cells are fibroblastic in morphology in culture dishes. However, they appear spherical with fine membrane extensions possibly supported by cytoskeleton in histological tissue sections. In the aged animals or injured specimens, NP cells transformed to either annular fibrosus like or fibroblast like. Studies have confirmed that ultrasound can stimulate the synthesis of ECM in human degenerative NP cells. Hence, the aim of this study is to study whether degenerative NP cell shape changes upon ultrasound and piezoelectric stimulation. Finally, we find that ultrasound and piezoelectric stimulation cause different effect on degenerative NP cells.

[Materials and Methods]

We used hydrogel to set up 3D cell cultures which mimic the *in vivo* substrate stiffness for NP cells. We utilized a biopsy punch to establish a stress/shear force model which may in part represents the mechanical stress on the NP cells in the process of IVD degeneration. Hydrogels ware seeded on either glass or quartz coverslips and stimulation was provided by Dish-LIC, an ultrasound stimulation chamber developed in the laboratory. The stimulation was continuous wave, and the voltage input was 12V.

[Results]

In the study, NP cells elongated tangentially along the edge of punctured site and transformed into oval shape within few days in control group. On the other hand, we observed that ultrasound and piezoelectric stimulation had opposite effect on the morphology of NP cells. To be more specific, ultrasound stimulation caused the cells to elongate more dramatically along the along the edge of punctured site. Conversely, piezoelectric stimulation significantly reduced the extent of NP cells elongation.

[Discussion]

Ultrasound and piezoelectric stimulation impacts the cell morphology in 3D hydrogel needle punctured model. To further investigate the therapeutic effect *in vivo*, we are designing an animal model of intervertebral disc degeneration for more elaborate experiments.

[Conclusions]

With ultrasound and piezoelectric stimulation on 3D hydrogel needle punctures model, we demonstrated that the morphology of degenerative NP cells can be effected by such such mechanostimulation.

Title of Project: Targeting Tissue Stiffness in Radiotherapy: Deciphering the Mechanism and Developing Treatment Strategies
Project No.: NHRI-EX113-11223EI
P.I. Name: Pai-Chi Li/李百祺
Key Professional Personnel: Jeng-Jong Hwang/黃正仲, Shao-Lun Lu/呂紹綸
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Shear Wave Elasticity Imaging Reveals the Impact of ECM Stiffness on Radiation Response of Liver Cancer: Overcoming Radioresistance through Sonoporation-aided LOX Inhibition in a Millimeter-Sized 3D Culture

Background:

Liver cancer, particularly hepatocellular carcinoma (HCC), often develops in a cirrhotic liver background, posing significant challenges for radiotherapy (RT). The presence of cirrhosis leads to a stiffer extracellular matrix (ECM) due to increased fibrosis, which can hinder the effectiveness of RT. Clinical observations have shown that tumors in a cirrhotic liver respond less favorably to RT compared to those in a non-cirrhotic liver. This reduced responsiveness is believed to be due to the mechanical properties of the ECM, which can affect the distribution and retention of therapeutic agents, as well as cellular responses to treatment. Lysyl oxidase (LOX) isoenzymes play a critical role in the cross-linking of collagen fibers, increasing ECM stiffness and contributing to the development of radioresistance.

Methods:

We constructed a 3D cell culture platform using a mixture of Matrigel and collagen, creating two stiffness conditions: normal (3 mg/ml collagen) and cirrhotic (4 mg/ml collagen), with corresponding shear moduli of approximately 1.5 kPa and 4.5 kPa. The cultures were treated with LOX inhibitor, BAPN (β-aminopropionitrile monofumarate), with or without sonoporation before embedding Huh7 human liver cancer cells into the matrix. Twenty-four hours post-gelation, the cultures were irradiated with 16 Gy photons and incubated for an additional 96 hours. Shear wave elasticity imaging (SWEI) was used to measure changes in ECM stiffness every 24 hours, and cell survival was assessed through flow cytometry. In our study, we used sonoporation-assisted LOX inhibition to investigate its impact on ECM stiffness and radiation response.

Results:

Increased ECM stiffness was associated with a decreased radiation response, indicated by the γ-H2AX and 7-AAD levels. LOX inhibition with BAPN significantly decreased initial ECM stiffness and increased RT-induced cell death. LOX inhibition was particularly effective in reducing ECM stiffness in stiffer matrices. Combining LOX inhibition with RT markedly increased radiation-induced DNA damage in cirrhotic liver cancer cells, enhancing their radiation-induced cell death. Furthermore, LOX inhibition combined with sonoporation effectively overcame stiffness-related radioresistance.

Conclusions:

The findings underscore the significant influence of ECM stiffness on liver cancer's response to radiation. Sonoporation-aided LOX inhibition emerges as a promising strategy to mitigate stiffness-related resistance, offering potential improvements in liver cancer treatment outcomes.

Title of Project: Evaluation of Chondrogenesis and Cartilage Repair via Second Harmonic Generation Imaging

Project No.: NHRI-EX113-11224EI
P.I. Name: Chung-Hwan Chen/陳崇桓
Key Professional Personnel: Shean-Jen Chen/陳顯禎, Chi-Hsiang Lien/連啓翔, Chun-Yu Lin/林俊佑
Affiliation/Institution: Kaohsiung Medical University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Background: Cartilage repair is a complex process, and clinically evaluating patients with knee cartilage repair is challenging. The major components of cartilage matrix are proteoglycans, collagen, and water. Native cartilage is composed of hyaline cartilage (type II collagen), while repair tissue is mainly fibrous cartilage (type I collagen). Identifying and quantifying fibrillar collagen without biomarkers is difficult due to the complex fibril assembly process. Currently, clinical evaluation methods like arthroscopy and MRI have limitations, as arthroscopy only provides surface images, restricting the assessment of cartilage composition quality. This project aims to develop a novel polarization-resolved imaging system for an intelligent cartilage collagen validation platform. This system enhances the understanding of collagen repair mechanisms at the molecular level, improving the quality assessment of repaired cartilage in tissue microenvironments.

Research approach and Results: Here, we have developed motion-free polarization control for SHG microscopy (PSHG) by utilizing a liquid crystal modulator (LCM) in the infinity space, allowing the generation of any desired linear and circular polarization state. Using a novel pixel-based polarization-resolved approach, we probed the net collagen α-helix pitch angle within gel mixtures. This approach also determined the SHG signal of collagen, revealing the peptide pitch angle for collagen gel matrices and articular cartilage from bone tissue.

Our study focuses on an explant culture system for porcine articular cartilage repair, employing PSHG microscopy to demonstrate changes in molecular and tissular scale structures with multi-parameters, such as peptide pitch angle (PA), anisotropy parameters (AP), anisotropy ratio, and SHG circular dichroism (SHG-CD). We found the effective pitch angles for collagen types I (Col I) and II (Col II) to be 49.7° and 51.6°, respectively. In the articular cartilage structure, from a depth of 0-500 μm, the average peptide PA value gradually decreases from around 49° in the superficial zone to 47° in the deep zone, then increases again to around 50° in the calcified cartilage zone.

We also obtained a series of polarization images illustrating variations in collagen mixture proportions by incorporating different concentrations of Col II into Col I hydrogel, ranging from 0% to 100%. Analysis of collagen during fracture healing revealed mean PA values of 49.26° at the 2-week mark and 49.05° at the 4-week mark. These findings enhance our understanding of collagen structure in cartilage repair and support the development of improved evaluation techniques.

Title of Project: 3-D Human Pancreatic Lesion Analysis: Duct, Islet, and Neurolymphatic Alterations in Inflammation
Project No.: NHRI-EX113-11225EI
P.I. Name: Shiue-Cheng Tang/湯學成
Affiliation/Institution: National Tsing Hua University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

The pancreas consists of both the exocrine acini and ducts (epithelium) and endocrine islets to facilitate and regulate digestive and metabolic activities. In humans, the acini, ducts, and islets hold direct contacts and form indirect neurovascular association to integrate the pancreatic structures and functions (*note*: unlike human islets, rodent islets are peri-lobular and enclosed by a glial sheath). Because the secretions of digestive enzymes and endocrine hormones are regulated by neuro-vascular signals, morphological assessment of human pancreases should include both the exocrine and endocrine components with neurovascular association to investigate the lobular structures in health and disease (e.g., diabetes or lesion formation). However, due to the dispersed and scattered nature of neurovascular tissues and islets, the clinical 2D histology cannot provide a global and integrated view to analyze the pancreatic tissue network in a 3D space continuum.

In this presentation, we will discuss our recent success of using the high-refractive-index (high-*n*) acrylamide-based polymer for human pancreas embedding and clearing to achieve antifade 3D/Airyscan super-resolution imaging (human liver as a parallel control to evaluate and avoid false positive and false negative results). In addition, we have integrated stereomicroscopy, clinical H&E histology, and in-depth super-resolution imaging to detect, confirm, and characterize the early and local remodeling of human pancreas (e.g., cystic change and low-grade duct lesion). We will use the unique duct-β-cell cluster as an example to illustrate the multimodal, multidimensional, and multiscale approaches of human pancreas imaging in the high-*n* polymer.

Recent publications on multimodal 3D/super-resolution human pancreas and liver histology

1. Tien YW, Chien HJ, Chiang TC, Chung MH, Lee CY, Peng SJ, Chen CC, Chou YH, Hsiao FT, Jeng YM, and **Tang SC***. Local islet remodelling associated with duct lesion-islet complex in adult human pancreas. ***Diabetologia***, 64:2266-2278, 2021. (official journal of European Association for the Study of Diabetes, EASD). *Cover image* on October issue.
2. Chung MH, Chien HJ, Peng SJ, Chou YH, Chiang TC, Chang HP, Lee CY, Chen CC, Jeng YM, Tien YW*, **Tang SC***. Multimodal 3-D/2-D human islet and duct imaging in exocrine and endocrine lesion environment: associated pancreas tissue remodeling. ***American Journal of Physiology - Endocrinology & Metabolism***, 323:E354-E365, 2022. American Physiological Society Journal. *Cover image* on October issue.
3. Hsiao FT, Chien HJ, Chou YH, Peng SJ, Huang TH, Lo LW, Sheng CN, Chang HP, Lee CY, Chen CC, Jeng YM, Tien YW, **Tang SC***. Transparent tissue in solid state for solvent-free and antifade 3D imaging. ***Nature Communications***, 14:3395, 2023.
   Highlight: In this paper, we use lesions in the human pancreas to illustrate 3D/Airyscan super-resolution imaging of tissue remodeling in a clinically related setting.
4. Chen CC, Peng SJ, Chou YH, Lee CY, Lee PH, Hu RH, Ho MC, Chung MH, Hsiao FT, Tien YW, **Tang SC***. Human liver afferent and efferent nerves revealed by 3-D/Airyscan super-resolution imaging. ***American Journal of Physiology - Endocrinology & Metabolism***, 326:E107-E123, 2024. American Physiological Society Journal. *Cover image* on February issue.
   Please visit www.3d-histology.com for visual summary of our work, including **1080p**^{HD} *videos.*

Title of Project: Development of a Modular Four-way Junction RNAi Scaffold Automatic Production and Packaging System for Targeted Multi-gene Silencing and Immune Checkpoint Blockade Therapy in Breast Cancer

Project No.: NHRI-EX113-11226EI
P.I. Name: Hung-Wei Yang/楊閎蔚
Key Professional Personnel: Dr. Hao-Han Pang/龐浩翰, Dr. Ying-Tzu Chen/陳映慈
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Breast cancer, recognized by the World Health Organization (WHO) as the most prevalent cancer worldwide, poses a significant threat to women's health. Among its subtypes, triple-negative breast cancer (TNBC) is considered the most malignant form. This study aims to develop a multifunctional platform for multi-RNA interference (RNAi) and immune therapy targeting TNBC. In the first year, we successfully prepared Fc-peptide fused virus-like particles (Fc@VLPs) encapsulating four-way junction (4WJ) RNAi to form Fc@4WJ-VLPs. The broccoli aptamer within the 4WJ RNAi scaffold allows rapid verification of the folding structure and concentration of the 4WJ RNAi scaffold within the Fc@4WJ-VLPs. In the second year of the project, we completed the structural analysis of Fc@4WJ-VLPs, efficiency analysis of antibody conjugation on the Fc@4WJ-VLPs surface, stability analysis of Fc@4WJ-VLPs, batch production stability analysis of Fc@4WJ-VLPs, cytotoxicity analysis of Fc@VLPs, cell uptake efficiency analysis of Fc@4WJ-VLPs, gene silencing efficiency analysis of Fc@4WJ-VLPs, and so on. Some key results are presented in Figure 1. Based on the outcomes of the second year, we will conduct further analysis in the third year of the project. This includes evaluating the gene silencing efficiency of delivered miR200, $siRNA_{c\text{-}MET}$, and $siRNA_{NF\text{-}\kappa B}$, and the associated effects on related pathways. Additionally, we will assess the effectiveness of $Ab_{PD\text{-}L1}$@4WJ-VLPs in immune checkpoint blockade therapy. Finally, we will perform preliminary animal experiments to verify the effectiveness of integrating multiple gene silencing therapy with immune checkpoint blockade therapy using $Ab_{PD\text{-}L1}$@4WJ-VLPs for TNBC treatment.

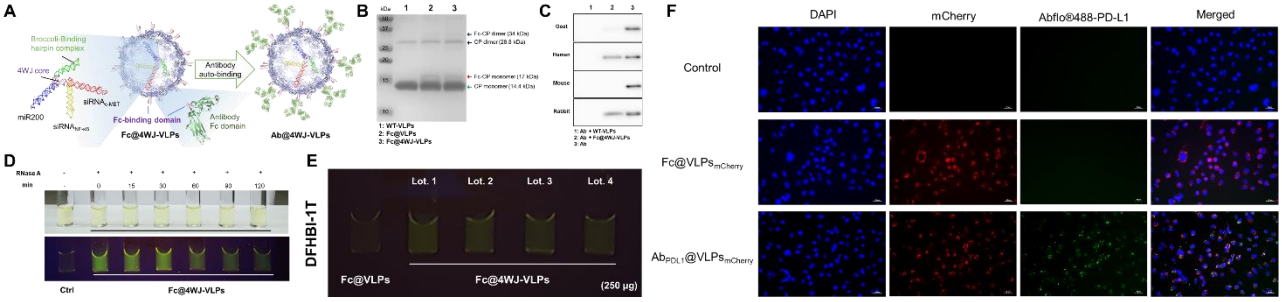


Figure 1. (A) Synthesis process of Fc@4WJ-VLPs with antibodies on the surface (Ab@4WJ-VLPs) via auto-packaging and antibody immobilization. (B) The 15% SDS-PAGE image showing WT-VLPs, Fc@4WJ-VLPs, and Ab@4WJ-VLPs. (C) Western blot analysis of PD-L1 antibody ($Ab_{PD\text{-}L1}$) mixed with WT-VLPs and Fc@4WJ-VLPs. The results demonstrated that only human-host and rabbit-host $Ab_{PD\text{-}L1}$ could bind to the surface of Fc@4WJ-VLPs. (D) Protection efficiency of 4WJ RNAi by Fc@VLPs. The results indicated that Fc@VLPs can effectively block RNase, preventing the degradation of the encapsulated 4WJ RNAi. (E) Batch production stability analysis of Fc@4WJ-VLPs based on the self-monitoring function of 4WJ RNAi. The results indicated that the quantity and quality of 4WJ RNAi in each batch of produced Fc@4WJ-VLPs are consistently uniform. (F) The cell uptake efficiency of Fc@4WJ-VLPs and $Ab_{PD\text{-}L1}$@4WJ-VLPs in MDA-MB-231 cells.

Title of Project: Novel Evaluation of Vestibular Functions for Clients with Cervicogenic Dizziness
Project No.: NHRI-EX113-11227EI
P.I. Name: Lan-Yuen Timothy Guo/郭藍遠
Key Professional Personnel: Lan-Yuen Guo/郭藍遠
Affiliation/Institution: Kaohsiung Medical University
Entire Project Period: From 2023 to 2025 (Total: 3 years)

Introduction: Clinical evaluation of patients presenting with dizziness could be differentiated by detecting eye movements in different criteria with eye trackers. Diagnosis of dizziness by observing oculomotor quality in single-plane head rotation is restricted by and the inability to achieve full-plane assessment of head positions with good accuracy, which may affect the results of oculomotor assessment. This study integrates a multi-axis Stewart platform with a desktop eye tracker (Gaze-point 3, GP3) for ocular motor analysis system. The purpose of this study was to assess inter-visit reliability of the ocular tracking ability parameters while applying different neck positions on pursuit task performance from a comprehensive perspective.

Methods: The study involved seven health participants (mean age: 28.52 ± 8.53 y/o), and used eye trackers: the GP3 (sampling rate: 60Hz) combined with Stewart platform. The subjects were positioned on the platform at a fixed distance from the screen, performing the pursuit of cyclic sinusoidal target movements in seven different neck positions (neutral, right/left rotation, right/left lateral flexion, and flexion/extension), with the platform passively twisting the trunk while maintaining the head still. Smooth pursuit parameters included gain, smooth pursuit neck torsion differences (SPNT-diff), phase error, and latency in different neck positions. The reliability of the data was verified with the intraclass correlation coefficient (ICC). Non-parametric tests were employed to assess the influence of neck positions on smooth pursuit function.

Results: The mean gain values exhibited a range of 0.54 to 0.72 in different positions. The ICC varied from 0.63 to 0.92, with the highest value recorded for the neck left rotation. The mean phase error values ranged from 5.81° to 10.19°, with the ICC varying from 0.64 to 0.95. The highest ICC was observed for the left lateral flexion. Latency values exhibited a range of 200.75 to 298 milliseconds, with the ICC varying between 0.08 and 0.85. The highest ICC was observed in neck flexion. The mean SPNT-diff values varied from 0 to 0.13. The SPNT-diff of flexion-extension exhibited the highest ICC value of 0.859. The statistical analysis indicated that SPNT-diff exhibited significant differences in the flexion-extension (p=0.03), whereas the other parameters did not show significant differences. In summary, the system demonstrated moderate or higher reliability in calculating smooth pursuit function parameters.

Conclusions: The results demonstrated a moderate to high degree of reliability in assessing comprehensive neck motion in different planes through integration with a multi-axial motion Stewart platform and an eye tracker, and offered the potential for a more comprehensive investigation of the mechanisms of oculomotor control for dizziness diagnosis.

Title of Project: Cold Atmospheric Plasma-Reinforced Micro/Nano-Biomimicked Hybrid Carrier Loaded with Platelet Lysate for Enhanced Osteoarthritis Attenuation

Project No.: NHRI-EX113-11323EI
P.I. Name: Er-Yuan Chuang/莊爾元
Affiliation/Institution: Taipei Medical University
Entire Project Period: From 2024 to 2026 (Total: 3 years)

Osteoarthritis (OA) can be partially managed with nonsteroidal anti-inflammatory drugs or hyaluronic acid, but these treatments only alleviate symptoms and do not halt cartilage degeneration. Platelet lysate (PL) offers a promising approach for healing or replacing damaged cartilage due to its high levels of growth and trophic factors. However, direct local administration of PL holds challenges such as rapid leakage, denaturation, short half-life of soluble factors, and risks associated with injections. Most studies overlook these intrinsic characteristics of PL and simply combine it with scaffolds.

A novel approach involves intraarticular administration of a micro/nano-PL carrier crosslinked by cold atmospheric plasma (CAP) using F127, glycol chitosan (GCS), and hyaluronic acid (HA). This method has shown improved material functionality, sustained drug release, and potential pathological tissue targeting, making it an effective delivery system for PL. This formulation is expected to enhance the efficacy of PL for treating OA. This study is the first to design a CAP-reinforced PL-based biomaterial, considering not only the intrinsic properties but also evaluating its bio-function and underlying mechanisms both *in vitro* and *in vivo*.

Title of Project: High-resolution AI Assisted Varifocal Endomicroscopy for in-vivo Brain Imaging Using Metalens

Project No.: NHRI-EX113-11327EI

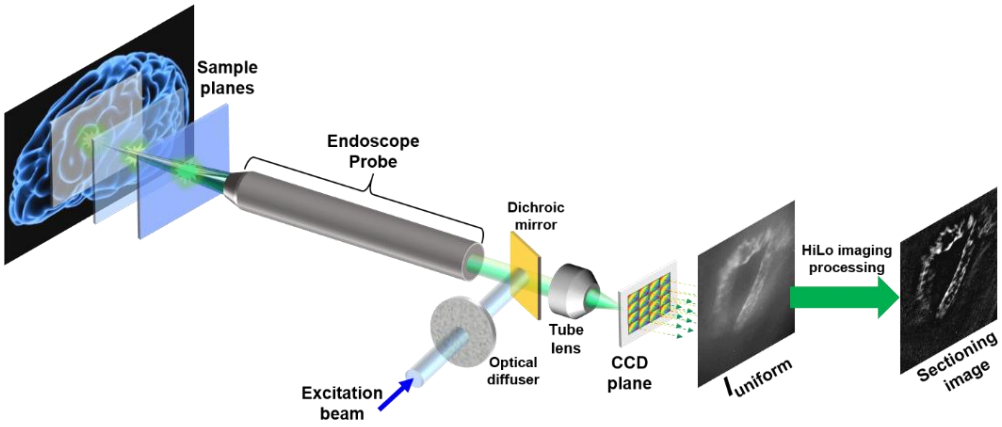
P.I. Name: Yuan Luo/駱遠

Key Professional Personnel: Yu-Hsin Chia/賈予鑫, Cheng-Hung Chu/朱正弘, Sunil Vyas, Chen-Yen Lin/林承彥, Pai-Yu Chen/鄭百諭, Min-Xuan Wang/王敏軒, Yi-You Huang/黃義侑, Yuan Luo/駱遠

Affiliation/Institution: National Taiwan University

Entire Project Period: From 2024 to 2026 (Total: 3 years)

Intracranial endo-microscopy has revolutionized neural science by enabling direct visualization of deep-seated brain areas. This study presents an innovative image reconstruction method for wide field endo-microscopy, capitalizing on the rapid acquisition capabilities and superior image quality of HiLo imaging. Through the application of histogram matching (HM) and a ResUNet model, the study aims to enhance intracranial visualization by aligning intensity profiles and preserving critical features. Evaluation results demonstrate a significant enhancement in Structural Similarity Index (SSIM) and Peak Signal-to-Noise Ratio (PSNR) metrics for the reconstructed images compared to the HiLo images. The PSNR values exceeding 30 dB and SSIM values surpassing 0.8 across all depths indicate the capability to achieve comparable quality to the HiLo system at various depths. These results highlight the effectiveness of the proposed approach in enabling high-quality real-time intracranial imaging outcomes.



Title of Project: Smart Care for Older Persons Recovering from Hip-fracture Surgery
Project No.: NHRI-EX113-10906PI
P.I. Name: Yea-Ing Lotus Shyu/徐亞瑛
Key Professional Personnel: Hsiu-Hsin Tsai/蔡秀欣, Chung-Chih Lin/林仲志, Wen-Ling Yeh/葉文凌, Chi-Chuan Wu/吳基銓, Su, Juin-Yih Su/蘇君毅, Huey-Shinn Cheng/鄭惠信, Jersey Liang/梁浙西, Ming-Yueh Tseng/曾明月, Yueh-E Lin/林月娥, Ming-Chin Yang/楊銘欽, Ying-Chao Chou/周應照, Ying-Jen Chen/陳英仁, Chen-June Seak/薛承君, Li-Chin Chen/陳麗琴
Affiliation/Institution: Chang Gung University
Entire Project Period: From 2020 to 2024 (Total: 5 years)

This clinical trial aims to develop and evaluate an innovative remote care model for older persons following hip fracture, known as the Smart Care Model, which utilizes smart clothing to improve caregiver competence in identifying fall risk and enable geriatric nurses to provide immediate feedback to enhance adherence to in-home rehabilitation. The Smart Care Model not only records daily activity levels but also detects body posture, including getting up, lying down, walking, and standing, by continuously monitoring the body's inclining angle. It can send warning signals, such as abnormal activity levels (too high or too low), abnormal body inclining angles, excessive nighttime mobility, prolonged periods of inactivity, unaccompanied outings, system malfunctions, and emergency calls, to home care nurses for timely intervention.

A randomized experimental design has been implemented in this study. The control group participants receive usual care, while the experimental group participants receive the Smart Care intervention. The subjects were recruited from the trauma wards of CGMH at Linkou and Tuchen, based on the following inclusion criteria: (1) age > 60 years, (2) admission to CGMH from the emergency department due to a one-side hip fracture, (3) received hip arthroplasty or internal fixation, (4) capable of performing full range-of-motion exercises against gravity and some or full resistance, with a pre-fracture Chinese Barthel Index score > 70, and (5) residing in northern Taiwan. A total of 158 subjects are planned to be recruited, with 79 in each group. The study outcomes will include patient outcomes (adherence, clinical outcomes, self-care ability, and health-related quality of life [HRQoL]) and family caregiver outcomes (preparedness, balancing competing needs, caregiver depressive symptoms, and HRQoL). The analyses will adhere to an intention-to-treat principle.

As of May 31, 2024, the fifth year of the study, 151 participant families have been enrolled, with 75 in the experimental group and 76 in the control group. There have been 4 (2 in the experimental group and 2 in the control group) attritions due to mortality and 14 (6 in the experimental group and 8 in the control group) dropouts. The recruitment of participants will continue, and the study will be completed during the remaining study period.
Key words: hip fracture, smart care, home nursing, older adults

Title of Project: Improving Care Coordination for Patients with Polypharmacy: The Development and Evaluation of a De-prescribing Program
Project No.: NHRI-EX113-11001PI
P.I. Name: Shou-Hsia Cheng/鄭守夏
Affiliation/Institution: National Taiwan University
Entire Project Period: From 2021 to 2025 (Total: 5 years)

Coordination of Care in a Health System Lacking Referral Arrangement: The Role of Patient Activation

Background. The prevalence of chronic conditions is rapidly growing among aged population and patient engagement and activation play a central role in health management. Patients with multiple chronic conditions tend to visit various physicians in different settings and receive poor coordinated care. While care coordination is crucial for high quality care and treatment outcome, several countries have introduced policies to improve coordination of care via primary care physician or gatekeeper. However, in many Asian countries without gatekeeper such as Taiwan, patients enjoy the autonomy and freedom of choosing preferred physicians. Whether patient activation is associated care coordination deserves investigation.

Objective. This study aimed to examine the relationship between patient activation and the patient- reported care coordination among older patients with chronic conditions.

Methods. A nationwide telephone interview survey of community-dwelling older adults was conducted in 2022. Subjects with at least one chronic condition and visited two or more doctors were included in the analysis (n=1237). Patient activation score was measured by Patient Activation Measures (PAM-10). The two care coordination questions asked whether the physician was aware of the patient's other medical conditions and visits to other physicians. Number of medications for chronic conditions was used as a proxy for multi-comorbidity.

Results. Patients with high or intermediate PAM scores were more likely to report better care coordination, namely doctor being aware of their other medical conditions (Intermediate: odd ratios [OR]: 1.44; 95% confidence interval [CI]:1.03-2.01; high: OR:1.93; 95% CI: 1.32-2.81) and their visits to other doctors (Intermediate: OR: 1.77; 95% CI:1.29-2.42; high: OR:2.34; 95% CI: 1.72-3.18). Similar results were also found in each dimension of PAM-10, including believes, knowledge, action, and staying the course under stress. Furthermore, we also found that the effects of patient activation were greater on care coordination for patients with multi-comorbidity.

Conclusion. Patient activation was strongly associated with care coordination, especially among patients with multiple chronic conditions. Improving activation of patients with comorbidities is the right direction in a health system lacking referral arrangement.

Title of Project: A Novel Multi-dimensional Prospective Study of the Gut-brain Axis through Metabolic MRI, Metabolomics and Gut Microbiome to Discover Gene-microenvironment Interactions in Neurodevelopmental Disorders

Project No.: NHRI-EX113-11002PI

P.I. Name: Susan Shur-Fen Gau/高淑芬

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Affiliation/Institution: National Taiwan University

Entire Project Period: From 2021 to 2025 (Total: 5 years)

Background: Autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD) are common neurodevelopmental disorders with significant long-term impacts. While distinct in their diagnostic criteria and interventions, they might share potential genetic influences and susceptibility factors affecting neuroanatomy, cognition, and behavior. However, simultaneous investigations of both disorders using consistent methodologies, particularly in metabolomics and microbiome studies, are rare. This five-year project aims to integrate gut-brain axis research, focusing on whole-body metabolism, to identify common and unique biomarkers and explore gene-microenvironment interaction mechanisms for ASD and ADHD.

Methods: This five-year prospective study collected various data including clinical symptoms/diagnoses, psychosocial functions, neuropsychology, neuroimaging (T1, T2, DTI, resting fMRI, MRS), metabolites (blood), and intestinal microbiome (stool) from 112 children with ASD, 120 children with ADHD, and 115 typically developing controls (TDC) aged 4-12 years, during the first three and a half years (Time 1). We integrated the multi-dimensional data and conducted experimental, image, multi-omic, and correlation analyses.

Results: After controlling age and sex, DTI analyses revealed higher fractional anisotropy values over the left corticospinal tract and corpus callosum forceps major in ASD, and lower fractional anisotropy values over the left inferior longitudinal fasciculus and corpus callosum tapetum in ADHD. MRS analyses found the ADHD group exhibited a higher choline to creatine (Cho/Cr) ratio in the left insula compared to the TDC group. Cutibacterium (genus level), Bacteroidaceae (family level), and Lachnospiraceae (family level) were identified in the ADHD group from microbiome analyses. We correlated the relative abundance of these three bacteria with the imaging results. We found positive association between mean diffusivity and the relative abundance of Lachnospiraceae, the negative association between mean diffusivity and the relative abundance of Cutibacterium, and a positive association between N-acetyl aspartate to Creatinine (NAA/Cr) ratio and the relative abundance of Cutibacterium. As for the ASD group, the relative abundance of Dialister succinatiphilus was negatively correlated with the fractional anisotropy of the left corticospinal tract. Additionally, negative correlations were identified between the abundance of Faecalibacterium longum and the genus Dialister with the Cho/Cr ratio in the right insula.

Discussions: We completed the data collection for Time 1 and have started to collect data for Time 2 (32 ASD, 35 ADHD, 23 TDC). Our preliminary results suggest both similarities and differences in brain and microbiome phenotypes between the two disorders, we also noted some gut-brain relationships. We will further establish gut-metabolite-brain relationships, and gut-brain-cognition/behavioral relationships. We aim to shed a light on the underlying mechanisms driving the pathogenesis of these disorders by examining whether these gut-brain axes deviate from typically developing controls (TDC) and differ between ASD and ADHD.

Title of Project: Sensory Phenotypes of Autism Spectrum Disorder Across Lifespan: Prospective Cohort Study and Sensory-Social Paradigm Establishment

Project No.: NHRI-EX113-11008PC
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Affiliation/Institution: National Taiwan University Hospital
Entire Project Period: From 2021 to 2024 (Total: 4 years)

Sensory characteristics have been recognized as one of core symptoms in autism spectrum disorder (ASD). Sensory symptoms may interfere social and adaptive function, as well as quality of life. Meanwhile, emotion recognition deficits represent a fundamental feature of ASD. This ability hinges on individuals' capacity to perceive emotional cues through visual observation. Previous research has indicated that individuals with ASD tend to focus on details and encounter challenges in processing visual information holistically. This perceptual style may imply impairments in integrating perceptual information in ASD. However, the specific impact of this visual perceptual processing style on facial emotion recognition and its neural underpinnings remains underexplored.

In the past 3.5 years, we have collected 450 autistic participants who completed the Sensory Profile. Among them, 107 completed follow-up questionnaires and were currently under statistical analysis. Initial analysis showed that atypical sensory symptoms remained at follow-up, while sensory sensitivity may decrease in a subsample of autistic participants.

For sensory-social paradigm, totally we recruited sixty individuals with ASD and sixty-two typically developing (TD) adults. Participants underwent a facial emotion recognition test in an MRI scanner. The test consisted of 216 faces categorized into 18 groups based on three spatial frequencies (Broad, High, Low) and six basic emotions (anger, disgust, happiness, neutral, sadness, surprise).

Preliminary analysis included the following. Firstly, compared to the TD group, the ASD group exhibited weaker activation in the left middle frontal gyrus (MFG) and right superior frontal gyrus (SFG) during tasks involving high versus broad spatial frequencies. Secondly, lower activation in the lingual gyrus, posterior cingulate cortex (PCC), and cuneus was found in the ASD group compared to the TD group when contrasting low spatial frequency with high spatial frequency conditions.

In conclusion, individuals with ASD demonstrated persisted sensory dysfunction at follow-up, that impact several domains of clinical symptoms. Besides, autistic adults displayed diminished activation in the posterior cingulate cortex (PCC) and cuneus, potentially impairing their ability to accurately identify and label emotions portrayed on faces, particularly in conditions that require processing without high spatial frequency information. This suggests that individuals with ASD tend to focus on details rather than process information holistically, which poses challenges in recognizing facial emotions.

Title of Project: Novel Brain Neurotechnology for Optimizing Precision Mirror Therapy in Stroke
Project No.: NHRI-EX113-11105PI
P.I. Name: Ching-Yi Wu/吳菁宜
Affiliation/Institution: Chang Gung University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

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Stroke remains a leading cause of adult disability, underscoring why research continues to focus on advancing new treatment methods and neurophysiological indexes While these studies may be effective, many lack a clear theoretical framework. The goal of the current study was to determine the optimal combination of mirror therapy and transcranial direct current stimulation (tDCS), and to evaluate its short-term and long-term effects on clinical outcomes. Additionally, we introduced electroencephalogram (EEG) indexes derived from the gating by inhibition model to explore the underlying therapeutic mechanisms. The EEG indexes used in this study focused on the functionally involvement for motor generation: alpha power at temporal regions (inhibiting non-motor activity) and central-frontal regions (releasing motor regions from inhibition). Firstly, post-training benefits, measured by Fugl-Meyer Assessment (FMA), was similar across 3 tDCS interventions (premotor, primary motor, sham). EEG seemed more sensitive to the training, with notable responses in the premotor tDCS group. Three months after training, only the premotor tDCS group maintained the gains in FMA, with these improvements also correlated with the EEG indexes—again, this pattern was specific to premotor tDCS. Since the gating by inhibition model suggests that EEG index reflect an individual's psychomotor efficiency, we also found that baseline EEG index could predict long-term FMA recovery. Our findings demonstrate the effectiveness of hybrid premotor tDCS and identify functionally oscillatory alpha-band activity in the temporal and central-frontal regions as potentially underlying the therapeutic mechanism. Notably, an individual's spatial pattern of EEG may be effective in predicting long-term upper extremity recovery.

Title of Project: Digital Dyadic Empowerment Program on Lifestyle Modification for Chronic Kidney Disease Management

Project No.: NHRI-EX113-11106PI
P.I. Name: Miao fen Yen/顏妙芬
Key Professional Personnel: Chun-Yi Ho/何俊毅
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Background: The long-term management of chronic kidney disease (CKD) requires active participation from patient-caregiver dyads in lifestyle modification. Digital interventions may offer scalable assistance to empower CKD dyads in modifying their lifestyles.

Objective: This study aimed to develop, test usability, and optimize a digital platform on the LINE instant messaging app, empowering CKD dyads in modifying their lifestyles based on the Digital Dyadic Empowerment framework (DDEF).

Methods: Utilizing Agile project management, the study followed a three-phase system development cycle. Phase 1 involved iterative platform development and trial use. After the prototype launch, 10 dyads tested the platform, providing feedback for optimization. Phase 2 consisted of a heuristic evaluation by 5 reviewers with relevant backgrounds, assessing compliance with Nielsen's ten usability heuristics and offering improvement suggestions. Phase 3 involved usability testing, inviting 5 dyads with experience using the digital platform to perform tasks and identify issues and possibilities for improvement in the platform usability.

Results: In Phase 1, the prototype of our platform, LINE Official Account (OA) "*Kidney Lifestyle*" and the extended App, launched after three months of iterative development. The platform offers functions such as health education, support resources, physiological data logging, reminder settings, and interactive features to promote lifestyle modification. A total of 19 participants (10 CKD patients and 9 significant others) participated in the platform trial use. Data and feedback from CKD dyads indicated continuous user engagement, high user acceptance and satisfaction. In Phase 2, heuristic evaluators generally found the functions complied well with the ten usability heuristics. Improvement suggestions included enhancing user experience, pushing more relevant and engaging information, and increasing interactive and feedback features. In Phase 3, results of usability testing suggested marginally acceptable usability for our platform. We observed that users experienced difficulty finding information within LINE OA, highlighting the potential to improve overall usability in this area. Results from each phase guided the next iteration to optimize our platform.

Conclusions: LINE Official Account "*Kidney Lifestyle*" and its extended App provide an innovative, instant-messaging-based platform supporting CKD dyads in lifestyle modification. The findings underscore its cost-effectiveness, wide availability, high user engagement, and potential clinical practicality. Future steps include conducting a feasibility study to assess implementation factors and potential clinical outcomes. This study contributes to the growing field of digital health interventions for CKD management by demonstrating the potential of a platform integrated with the LINE instant messaging app.

Title of Project: The Effect of Early Life Exposure to Emergent Environmental Pollutants on Child Development: A Cohort Study Based on Taiwan Southern Human Milk Bank
Project No.: NHRI-EX113-11116PI
P.I. Name: Yung-Chieh Lin/林永傑
NHRI Researcher: Po-Chin Huang/黃柏菁
Key Professional Personnel: Pao-Lin Kuo/郭保麟, Yu Tsung/余聰
Affiliation/Institution: National Cheng Kung University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Background/Study Aims: Phthalates and its new substitutes, a group of new emergent environmental plasticizers (NEEP), have been reported to be endocrine disrupting chemicals. Plasticizers is shown to impact the health of pregnant women, human fetuses, and infants, and has been extensively researched. However, scant studies have looked specifically at the NEEP on those vulnerable group. Human milk has been recognized as the main source of nutrition for infants and has been linked with long-term health of infants. Human milk might be a overlooked route of NEEP passing from maternal exposure to neonatal exposure. Through longitudinal maternal and infantile blood, urine, and human milk analysis from trimester to postnatal of life, this study sought to assess the exposure of NEEP and the long-term hormonal disrupting impact on the maternal health and the neurodevelopment of young infants.

Methods: As a 4-year prospective cohort study in 2022–2025, 600 maternal participants would be recruited in 4 years and contacted by a case manager from the obstetric outpatients clinics, obstetric ward, baby ward, and Taiwan southern milk bank located in a university tertiary hospital. Maternal blood, urine of mother-baby dyads, and human milk were collected from antepartum to early postpartum stage. Maternal blood was tested for thyroid function test (TFT), including total thyroxin (TT4), triiodothyronine (T3), free thyroxin (FT4), free triiodothyronine (FT3), and thyroxine-binding globulin (TBG). Urine and human milk were investigated for NEEP concentration at NHRI. Questionnaires, medical history, physical examination, and anthropometry was recorded by a medical team.

Results: In the 2.5 years, maternal participants (N=355) were recruited, and their infants dyads (N=175) was jointed after the labor. Urine sample (n=397) from 197 maternal participants and urine sample (n=150) from 133 infant dyads was analyzed. Human milk sample from mothers (n=134) and maternal thyroid function test (n=150) were studied. Perinatal urine phthalates metabolites concentrations were obtained from 197 maternal participants, and the mean[median] (ng/ml) of each metabolites were: MMP 24.7[17.2], MEP 109[27.5], MiBP 11.5[6.1], MnBP 22.1[9.2], MBzP 2.3[0.0], MEHP 9.0[0.0], MEHHP 18.4[9.9], MEOHP 20.6[14.0], MECPP 16.9 [10.4], MCMHP 6.3 [3.9], and MiNP 3.1 [0.0]. One of 150 TFT showed intermediate TSH. Only T3 showed significantly associated with MnBP (Pearson correlation=0.167; p=0.048) in perinatal stage. Meanwhile, urine phthalates metabolites concentrations were obtained from 133 infant participants, and the mean[median] (ng/ml) of each metabolites were: MMP 21.9 [7.2], MEP 105[29.4], MiBP 12.9[5.4], MnBP 14.5[5.1], MBzP 15.1[0.0], MEHP 46.8[0.0], MEHHP 21.2[4.2], MEOHP 25.1[4.7], MECPP 98.4 [10.4], MCMHP 3.9 [0.0], and MiNP 1.5 [0.0]. Without adjusted daily milk intake, the infant urine phthalates metabolites concentrations did not correlated with respective metabolites concentration in their mother's milk. However, the urine phthalates metabolites concentration difference between mother-infant-dyads were only significant in MEHP(infant>mother; p=0.028).

Conclusion: This study preliminarily showed urine phthalates metabolites of infants are similar level to their mother's urine phthalates metabolites, even, urine MEHP of infants is higher than that of their mothers. NEEP could pass from maternal exposure to fetal neonatal exposure should be cautious to women during pregnancy. The long-term follow-up on the health of infants is warranted.

Title of Project: Interactions between Host Immunogenetic Variants and Epstein-Barr Virus Antibody Responses on the Risk for Nasopharyngeal Carcinoma: A Large-Scale Case-Control Study
Project No.: NHRI-EX113-11117PI
P.I. Name: Mei-Hsuan Lee/李美璇
Key Professional Personnel: Yi-Ting Chen/陳翊霆, Szu-Ching Yin/尹思晴, Wan-Lun Hsu/徐婉倫, Chien-Jen Chen/陳建仁, Cheng-Ping Wang/王成平
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Nasopharyngeal carcinoma (NPC) is a rare cancer that exhibits familial clustering. In Taiwan, the incidence of NPC is relatively high compared to other countries. Epstein-Barr virus (EBV), which infects more than 90% of adults worldwide, is a well-known determinant contributing to the development of NPC. However, the extent to which genetic alterations influence EBV control and its association with NPC remains incompletely understood. The human leukocyte antigen (HLA) region, known for its complex nature within the human genome, plays a crucial role in immune responses to clear foreign antigens. Despite extensive research on EBV and HLA, the interactions between host genetic variants and virus controls in relation to NPC have received limited attention. Therefore, we proposed a large-scale case-control study involving 1,998 NPC cases and 2,131 unaffected controls. The aim of this study is to investigate the associations of *HLA* variants and the interplay between specific *HLA* alleles and EBV antibody-based signatures in determining the risk of developing NPC. From Jan 2022 to May 2024, we have performed 2235 samples (1040 NPC cases and 1195 controls) for whole-genome SNP array (TWB 2.0) which includes 686,389 variants in the human genome. Among the 2235 samples, 1653 (828 NPC cases and 825 controls) were successfully genotype and passed the quality control. All of these samples were performed for subsequent analyses. There were 74% (1220/1653) males; the mean age was 47.8 years old. The blood samples were collected and tested for IgA antibodies against EBV viral capsid antigen (VCA) and nuclear antigen 1 (EBNA-1). Amongst total samples, 34% and 26% were seropositive for EBNA-1 and VCA IgA, respectively. More NPC cases were seropositive for either EBNA-1 and VCA IgA, by comparing to unaffected controls ($p<0.001$). We may impute the 8 major HLA genotypes, including class I (*A*, *B*, and *C* locus); and class II (*DPA1*, *DPB1*, *DQA1*, *DPB1*, and *DRB1*). We compared the allele frequencies for NPC cases and controls. The study will narrow down to specific variants from the complex *HLA*, which is helpful for immunological studies on investigating the mechanisms of antigen presentation and immune regulations. It will provide more insights for NPC pathogenesis as well as future EBV vaccine development. We plan to finish the remaining samples by next year then we may perform imputation to investigate host and virus interactions on the associated risks for NPC development.

Title of Project: A Learning Health System Integrating Clinical and Genomic Information to Enable Early Detection and Early Intervention for Children with Developmental Delay/Intellectual Disability

Project No.: NHRI-EX113-11118PI
P.I. Name: Yann-Jang Chen/陳燕彰
NHRI Researcher: Shih-Feng Tsai/蔡世峯
Key Professional Personnel: Sung-Hui Tseng/曾頌惠; Ming-Lan Tsai 蔡明蘭
Affiliation/Institution: National Yang Ming Chiao Tung University
Entire Project Period: From 2022 to 2025 (Total: 4 years)

Developmental delay and intellectual disability (DD/ID) affect 5-7% of children. In Taiwan, there is already a government-supported system for screening children with DD/ID and providing early intervention, which includes medical and educational support. However, clinicians often face frustration because a genetic etiologic diagnosis frequently does not lead to therapeutic treatment for children with DD/ID. This can seriously undermine patient trust in science and healthcare.

In our project, we aim to establish a nationwide learning health system fostering a continuous learning and improvement cycle. This system will enhance the identification of specific patient groups and the design of optimized management plans for individual patients. Our aims are **Aim 1**: to establish a national network for integrating clinical phenotype, multidisciplinary evaluation data, and genomic information. **Aim 2**: to combine short-read sequencing and long-read sequencing technology for the detection of causative structural variants in DD/ID. **Aim 3**: to achieve patient stratification and personalized management (especially those with epilepsy phenotypes) for DD/ID. **Aim 4**: to investigate the clinical utility of genomic tests for early intervention through health technology assessment. **Aim 5**: to conduct a transethnic comparative genomics study on DD/ID through international collaboration

Till now, we have finished the following.

1. We have established a database platform including all sequencing and phenotypic raw data of all enrolled subjects and provided a user-friendly interface for collecting essential data elements necessary for diagnosing DD/ID.
2. We used Williams syndrome patients to test and compare two main genomic sequencing techniques, short-read and long-read sequencing. We have finished 15 samples of WGS by long-read sequencing (PacBio) and short-read sequencing (Illumina). We will then apply the long-read sequencing technique to detect unknown-causing DD/ID patients after short-read sequencing technique from our enrolled subjects.
3. We have recruited 92 children with DD/ID and performed WES NGS analysis. There are 30 females and 62 males. Their age ranged from 9 months old to 18 years old. Ninety cases have finished WES analysis and the others are ongoing. Positive detection was around 30-40%.
4. We are currently actively engaging with genomic centers in Japan and the UK. By organizing relevant international conferences, we aim to foster exchange and collaboration, hoping to achieve cross-ethnic genomic comparisons and research in the future

We will continue to collect DD/ID cases and conduct genetic abnormality analyses. Additionally, we will optimize our database to facilitate reanalysis and integration. At the same time, we will utilize new technologies to improve the detection rate of DD/ID patients and develop new treatment protocols, such as using growth hormone therapy for DD/ID patients with specific genetic variants, to enhance overall healthcare for DD/ID patients. We will also strengthen cooperation with international institutions to advance genomic testing capabilities in Taiwan.

Title of Project: Applying Smart Health Technology and Precision Medicine to Facilitate the Delivery and Documentation of High Quality Cardiopulmonary Resuscitation

Project No.: NHRI-EX113-11137PI

P.I. Name: Tsung-Chien Lu/呂宗謙

Key Professional Personnel: Chih-Hung Wang/王志宏, Fan-Ya Chou/周凡雅, Feipei Lai/賴飛羆

Affiliation/Institution: National Taiwan University Hospital

Entire Project Period: From 2022 to 2024 (Total: 3 years)

To Develop a CPR Feedback and Monitoring Device Using Smart Health Technology to Be Used During Resuscitation Events

Introduction: Cardiopulmonary resuscitation (CPR) quality affects the prognosis from cardiac arrest. Current guidelines emphasize on compressions of adequate depth of 5-6 cm and rate of 100-120/min. **Objectives**: In this study, we aimed at creating a highly mobile feedback device with the algorithms we previously developed by using a smartwatch (ASUS ZenWatch 2) with sensor data from its accelerometer during chest compression on a manikin, which is capable of detecting the rate and depth of CPR during resuscitation and providing real-time feedback. **Methods**: We created a prototype of a CPR feedback device featuring an embedded accelerometer and advanced algorithms enabling real-time prediction of CPR quality in terms of chest compression rate and depth. This innovative prototype is seamlessly integrated with a Raspberry Pi model 4 acting as a signal transducer computing between the device and a laptop PC. It was then used to conduct a validation study to assess the accuracy of chest compression rate measured by the device and the manikin while we perform chest compression only CPR by researchers. In the validation phase, two researchers serving as rescuers executed 2-minute sessions of chest compression-only CPR on the Resusci Anne QCPR training manikin across various target sessions. Each researcher conducted five distinct sessions, contributing to a total of 10 sessions. Using the Laerdal PC SkillReporting software, we recorded and analyzed the associated rate and depth data of CPR performed on the manikin. We compared the chest compression depth predictions generated by our prototype, featuring previously developed algorithms, with the reference data from Resusci Anne. By using Bland-Altman (BA) analysis, we assessed the agreement on feedback between our methodology and the reference method. **Results**: By using BA analysis, the mean difference of depth between our method and the reference standard was -0.011. The bias between the two methods was not significant, with a 95% confidence interval of -0.1083 to 0.1295. Further Bland-Altman analyses on subgroup analysis were performed based on different target rates: 80 to 99, 100 to 120, and 121 to 140. The mean differences of depth for these target rates were 0.018, 0.003, and -0.046, respectively. with 95% confidence intervals of -0.2104 to 0.1741, -0.2135 to 0.2071, and -0.1624 to 0.2541, respectively. **Conclusion**: By using the novel algorithms that we developed, we successfully validated that these algorithms can be accurately predict chest compression depth when embedded on a prototype of CPR device we developed in this project, which will be used to conduct a randomized control trial in clinical setting.

Title of Project: Environmental Co-exposure to Melamine and Phthalates and the Risk of Kidney Injury in Schoolchildren

Project No.: NHRI-EX113-11202PI
P.I. Name: Ming-Tsang Wu/吳明蒼
NHRI Researcher: Chu-Chin Chen/陳主智
Key Professional Personnel: Shu-Li Wang/王淑麗, Mei-Lien Chen/陳美蓮, Chia-Jung Hsieh/謝佳容, Chia-Fang Wu/吳佳芳, Chih-Hsing Hung/洪志興, Fu-Chen Kuo/郭富珍, Hui-Ju Tsai/蔡惠如
Affiliation/Institution: Kaohsiung Medical University
Entire Project Period: From 2023 to 2027 (Total: 5 years)

Background: Melamine and phthalates are two common and potential environmental nephrotoxins. The study to investigate the interactive effect of melamine and phthalates on kidney function in children is scarce. This study aims to examine the individual and interactive effect of exposure to melamine and phthalates from either children themselves or their mothers during pregnancy on early markers of renal injury in children. The sex-specific difference in the above-mentioned relationship is also explored.

Methods: The total of 1,676 pregnant women were enrolled in the original Taiwan Maternal and Infant Cohort Study (TMICS), a multicenter prospective birth cohort study, between 2012 and 2015. Their offspring, when reached three years and older, was recruited between August, 2016 and February, 2020 and one-spot overnight urine specimens were collected to simultaneously measure melamine, 11 phthalate metabolites, and two markers of renal injury, microalbumin and N-acetyl-beta-D-glucosaminidas (NAG). Estimated daily intakes of melamine and six phthalates, including DEHP (di-2-ethylhexylphthalate), DiBP (Dibutyl phthalate), DnBP (Di-n-butyl phthalate), BBzP (Butyl benzyl phthalate), and DEP (Diethyl phthalate), were estimated using a creatinine excretion-based model from urine melamine and phthalate metabolites in children and their mothers during pregnancy at third trimester.

Results: Five hundred fifty-two children were studied. The mean age was 4 years; 319 (57.8%) were boys. Median estimated daily intakes (μg/kg bw/day) were 5.73 for DEHP, 1.75 for DEP, 3.23 for DnBP, 0.07 for BBzP, 1.37 for DiBP, and 1.18 for melamine; there is not significantly different between boys and girls. Boys in the highest quartile of estimated melamine intake (≥ 0.68 μg/kg/day) had a significantly higher urine ACR levels and in the highest quartile of estimated phthalate intake of DEHP (≥ 5.36 μg/kg/day), DEP (≥ 0.89 μg/kg/day), and DiBP (≥ 1.19 μg/kg/day) had significantly higher urine NAG levels, when compared to the combined three lowest quartile ones in their comparison groups. By contrast, the same significant results were not found in girls. There is also no significant relationship of their mothers' phthalates and melamine intake during pregnancy at third trimester (n = 438) with renal injury markers in children. Categorized the highest quartile of the above four significant chemicals as "1" score and summed, the higher the score of the joint effect of melamine, DEHP, DEP, or DiBP, the higher urine ACR and NAG levels, particularly in boys.

Conclusions: Boys with high exposure of melamine and certain phthalate chemicals (DEHP, DEP, and DiBP) have increased markers of early kidney damages. A longitudinal follow-up study is necessary to elucidate the causality of the two common environmental hazards on adverse kidney function in children.

Title of Project: Decision Analysis of Care and Prevention of Chronic Kidney Disease : Establish a Model to Support Sustainable Health Goals

Project No.: NHRI-EX113-11208PI
P.I. Name: Ming Yen Lin/林明彥
NHRI Researcher: Chih-Cheng Hsu/許志成
Key Professional Personnel: Yi-Wen Chiu/邱怡文, Shang-Jyh Hwang/黃尚志, Ping-Hsun Wu/吳秉勳, Cheng-Yin Chung/鍾承穎, Lii-Jia Yang/楊禮嘉, Yihuang Kang/康藝晃, Jeng-Huei Chen/陳政輝, Hsing Luh/陸行
Affiliation/Institution: Kaohsiung Medical University Chung-Ho Memorial Hospital
Entire Project Period: From 2023 to 2026 (Total: 4 years)

Background: Taiwan has top end-stage kidney disease (ESKD) incidence and prevalence in the world. The government has initiated pre-ESRD and early chronic kidney disease (CKD) care programs to slow the rapidly increasing number of cases requiring kidney replacement therapy. The optimal CKD care models for patients with different CKD stages remain unknown. Therefore, the four-year project aims to develop decision support models to assist the government, people, and caregivers make appropriate decisions to promote a sustainable kidney health system.

Materials and Methods: We developed models to understand CKD state transitions and their relevant factors through the Health and Welfare Data Science Center, Ministry of Health and Welfare (No: H112018). People who participated in the Taiwan Adult Preventive Health Service program from 2012–2020 were study subjects. We integrated their time-sequential values of estimated glomerular filtration rate (G1: >90 to G5: <15) and urine dipstick (A1:"-" to A3: "≥1+") into G1A1 to G5A3 states. ESKD and death information were obtained from the catastrophic illness and the national death registry databases, respectively. We fitted the follow-up time by Weibull distribution for each state transition. The cause-specific Cox model incorporated state-specific demographic factors, life habits, comorbidity, and metabolic syndrome scores to understand their associations with state-specific hazards.

Results: A Total of 4.7 million times state transitions occurred in 15.7 million screening visits. After fitting the Weibull distribution for the follow-up time, the mean shape value was 1,373 for those aged <65 and 772 for those aged ≥ 65. The mean scale value of the Weibull distribution was 2.75 for those aged <65 and those aged ≥ 65. The median follow-up time from CKD state to death gradually decreased with a more advanced CKD state from 13.9 in G1A1 to 1.9 in G5A3 and a little bit extending to 2.8 years in ESKD. For example, the hazard ratio from G1A1 for people with diabetes had 1.07 to G1A2, 1.45 to G1A3, 0.9 to G2A1, 1.01 to G2A2, 1.41 to G2A3, 1.47 to G3aA1, 1.67 to G3aA2, 2.5 to G3aA3, 2.09 to G3bA1, 2.85 to G3bA2, 3.68 to G3bA3, 2.66 to G4A1, 0.57 to G4A2, 3.81 to G4A3, 1.23 to G5A1, 0.85 to G5A2, 2.93 to G5A3, 4.16 to ESKD, and 1.43 to death compared with people without diabetes after controlling other selected factors.

Challenges: Only a tiny case number in more advanced CKD states due to the screening program characteristic makes the model parameter estimation less reliable.

Keywords: chronic kidney disease, natural history, transition, probability, hazard, factors

Title of Project: Older Volunteers' Competence Assessment and Training for Community-based Long-term Care Services

Project No.: NHRI-EX113-11209PI
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Key Professional Personnel: Jing-Jy Wang/王靜枝, Li-Hui Lin/林麗惠, Tzu-Yu Lin/林子郁, Meng-Chin Chen/陳孟勤, Chiang-Ching Chang/張江清
Affiliation/Institution: Kaohsiung Medical University
Entire Project Period: From 2023 to 2026 (Total: 4 years)

Background: Older volunteers are the main human resource in community care centers of Taiwan. However, there is no sufficient and tailored training program for older volunteers to enhance their competencies in providing community-based long-term care services. It is essential to identify core volunteer competency indicators and to provide corresponding training programs to enhance their knowledge and skills in providing services.

Purpose: The first and second years of this project aimed to identify the competencies of older volunteers that are needed to provide community-based long-term care services, and to develop a scale for assessing the competencies and needs of older volunteers and to establish its psychometric properties

Methods: The 1st year study comprised two phases. In phase I, two focus group interviews were conducted with 12 experts to identify the core competency indicators of older volunteers. In phase II, a preliminary "Older Volunteer Competency Scale" was developed and finalized by 12 experts after two rounds of the Delphi panel's reviews, critiques, and modifications. In the 2nd year, we are utilizing survey research to assess the volunteers' competency and needs, to further establish the scale's psychometric properties.

Results: In phase I, 12 experts were invited to participate in two focus group interviews with 6 experts in each group (practitioners vs. scholars). The experts aged between 41 and 71 years (*M* = 61.75; *SD* = 8.44). Majority of them were female (*n* = 8; 66.7%), had master's or above education (66.7%), and had an average of 16.33 years (*SD* = 2.78) of experience working with older volunteers, including (1) 2 geriatric nurses, (2) 2 geriatric social workers, (3) 2 geriatric educators, (4) 3 senior activity center directors, and (5) 3 volunteer leaders of the senior activity centers. In phase II, 12 domain experts were invited to join the Delphi expert group to further evaluate item validity. The 12 experts aged between 41 and 77, had a mean age of 61.33 (*SD* = 10.04). Most of them were female (58.3%), had an average of 19.83 years (*SD* = 8.56) of experience working with older volunteers, and had master's or above education (66.7%). Their backgrounds included: (1) 2 geriatric nurses, (2) 2 geriatric social workers, (3) 2 geriatric educators, (4) 2 senior activity center directors, (5) 2 volunteer work leaders, and (6) 2 older volunteers of the senior activity centers. In the first round of Delphi, the I-CVIs on the item importance, appropriateness, and clarity were higher than 0.75 (S-CVI/Ave = 0.96; S-CVI/UA = 0.64), 0.75 (S-CVI/Ave = 0.93; S-CVI/UA = 0.36), and 0.75 (S-CVI/Ave = 0.92; S-CVI/UA = 0.36), respectively. In the second round of Delphi, the scores for item importance, appropriateness, and clarity were all 1.0 (S-CVI/Ave = 1.0; S-CVI/UA = 1.0). The finalized "Older Volunteer Competency Scale" has 35 items measuring three constructs: (1) service awareness (10 items), (2) service skills (18 items), and (3) interpersonal interaction (7 items).

Conclusion: The instrument "Older Volunteer Competency Scale" developed in the first year has been used as an assessment tool in the second year of the study to assess the volunteers' competency and needs. Apart from establishing the scale's reliability and validity, the scale will also be utilized to facilitate the planning of subsequent training courses for older volunteers.

Title of Project: Scale-out of a Home-based Arm and Hand Exercise Program for Stroke: A Multisite Implementation-efficacy Trial

Project No.: NHRI-EX113-11210PC

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Affiliation/Institution: Chang Gung University

Entire Project Period: From 2023 to 2026 (Total: 4 years)

Introduction/Rationale: The Graded Repetitive Arm Supplementary Program (GRASP) is an evidence-based exercise program designed to improve upper extremity function in individuals with stroke. No study has been conducted to explore the perspectives of individuals with stroke who receive the GRASP program.

Objectives: To explore the experience and acceptability of the Graded Repetitive Arm Supplementary Program (GRASP) for individuals with stroke from the patients' perspective.

Method or Approach: This qualitative study uses semi-structured interviews relating to the perspectives of the GRASP program. Ten patients with stroke participated in the experimental arm of an effectiveness-implementation hybrid trial that had a dual focus: the effectiveness of the intervention on clinical outcomes and implementation outcomes.

Results or Practice Implications: Four themes were identified relating to the experience and acceptability of the GRASP program: (1) "Motivations matter" highlighted that motivations affect how patients implement the GRASP program; (2) "GRASP is acceptable" described that GRASP was acceptable by patients; (3) "GRASP is adjustable" emphasized the GRASP exercises are easily modified and progressed based on the principles of GRASP and therapists' clinical experiences; (4) "GRASP improves upper extremity motor function" outlined the improvement in arm and hand motor function after patients received the GRASP program.

Conclusion: Narratives from people with stroke provided insight into the experience and acceptability of the GRASP program. Future studies examining the experience and acceptability of evidence-based interventions are warranted to guide the uptake of evidence-based interventions.

Title: Implications and Ramifications of the CDC Tier 1 Genetic Screening Concept in East Asian Populations

P.I. Name: Dr. Shih-Feng Tsai/蔡世峯
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Institute/Center: National Health Research Institutes

The CDC's office defines the Centers for Disease Control and Prevention Tier 1 (CDCT1) genomics applications, advocating mass screening for three hereditary disease conditions with significant potential impacts on public health. Two recent cohort studies in the U.S. have investigated a range of genetic risks by screening for hereditary breast and ovarian cancer syndrome (HBOC), Lynch syndrome (LS), and familial hypercholesterolemia (FH). With the CDCT1 genetic screening concept, we integrated two Taiwanese population-specific genes, *NOTCH3* and *GJB2*, which were related to Cerebral Autosomal Dominant Arteriopathy Subcortical Infarcts and Leukoencephalopathy (CADASIL) and hereditary hearing loss into our study. However, the genetic risks associated with these relatively common medical conditions and their screening yields remain unknown for the Taiwanese population and East Asian countries.

We analyzed the whole genome sequence (WGS) data of 1,454 participants from the Taiwan Biobank (TWB) and 2,473 participants from the National Health Research Institutes (NHRI) cohort to investigate pathogenic and likely pathogenic (P/LP) genetic variants of the CDCT1 genes, *NOTCH3*, and *GJB2*, as annotated by SnpEff, ClinVar, and VarSome.

In the TWB and NHRI cohorts, we found a combined carrier rate of 1.44% and 1.09% for P/LP genetic variants of HBOC, LS, and FH; 0.75% and 0.89% for CADASIL; 18.36% and 19.53% of hereditary hearing loss. Among these carriers, we identified 10 P/LP variants of *BRCA2, MSH2, APOB, LDLR, NOTCH3,* and *GJB2*, with allele frequencies higher than 0.1%. Comparing our results with those available from the gnomAD, SG10K, and TogoVar databases, we found that five variants (*BRCA2*, c.8954-5_8954-2del; *MSH2*, p.Arg929Ter; *ApoB*, p.Arg3527Tep; *GJB2*, p.Val37Ile, and p.His100ArgfsTer14) showed higher AF in individuals of Chinese and Malay ethnicity, and other five variants (*BRCA2*, p.Ala2786Thr; *MSH2*, p.His839Arg; *LDLR*, p.Asp90Asn; *NOTCH3*, p.Arg544Cys; *GJB2*, p.Leu79CysfsTer3) were only present in individuals of Chinese ethnicity. Furthermore, using the Healthy Aging Longitudinal Study in Taiwan (HALST) and the TWB long-term cohorts, we have identified carriers of FH variants and confirmed that they have higher risks in cardiovascular disease and total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels than the average. Only 40% of these individuals took the cholesterol-lowering medication in the HASLT cohort, and 37.5% were unaware of FH in the TWB cohort.

Our study represents the first adoption of CDCT1 genetic screening in Asia and proves its portability and ramifications to East Asian populations. Taking a transethnic comparison, we have identified population-specific variants that can be used to target at-risk individuals who might benefit from early detection and timely treatment.

Title of Project: Outcomes of Mirror Therapy Preceding Augmented Reality in Stroke Rehabilitation
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Affiliation/Institution: Chang Gung University
Entire Project Period: From 2024 to 2026 (Total: 3 years)

Background: Mirror therapy (MT) and augmented reality (AR) are gaining popularity in stroke rehabilitation. MT utilizes mirror visual feedback to promote bilateral brain coupling and increase excitability of the motor areas. AR offers an interactive context of gamified practice for promoting motor, balance, and cognitive recovery. MT and AR may complement each other for hybrid interventions in stroke rehabilitation. This poster presented preliminary findings of the efficacy study of AR with and without the priming of MT, relative to dose-matched control treatment (CT) in stroke rehabilitation.

Method: Thirty-six first-ever unilateral stroke patients were randomly assigned to three groups: MT+AR, AR, and CT. Each session lasted 90 minutes, three times a week for six weeks. The MT+AR group received 40 minutes of MT, 40 minutes of AR, and 10 minutes of functional task practice. The AR group had 80 minutes of AR and 10 minutes of functional practice, while the CT group had 80 minutes of therapist-mediated occupational therapy followed by 10 minutes of functional practice. Home practice involved 30 minutes practice of functional activities, five days a week. Assessments were conducted pre-test, post-test, and after 3-month follow-up. Primary outcome measures were the Fugl-Meyer Assessment-Upper Extremity (FMA-UE) and Berg Balance Scale (BBS). Secondary outcome measures included revised Nottingham Sensory Assessment (rNSA), Chedoke Arm and Hand Activity Inventory (CAHAI), Functional Independence Measure (FIM), and Stroke Impact Scale Version 3 (SIS). Adverse events were monitored before and after the intervention.

Results: The participants in the three groups matched in baseline characteristics. The MT+AR and AR group improved in primary and secondary outcome measures immediately after the interventions. The CT group had limited improvement in rNSA. The MT+AR group demonstrated greater improvements in FMA-UE and CAHAI scores and showed significantly better progress in rNSA compared to both the AR group and the CT group. The AR group demonstrated significantly better improvements in BBS than the other groups. Both the MT+AR and AR group improved more than the CT group in life quality as measured by the SIS. There were no significant differences in change in FIM scores among the groups. There was no adverse response during the study period.

Conclusion: Both MT+AR and AR effectively enhanced sensorimotor and balance function, bilateral upper-limb function, independence of daily activities, and quality of life in stroke patients with moderate to severe motor impairments. Both groups showed differential benefits. MT+AR was more effective in improving upper limb motor function and sensory function, while AR led to greater improvement in balance and functional mobility. Further study is needed to identify the factors that may affect retention of treatment gains over time.

Keywords: Stroke, Mirror therapy, Augmented Reality, Gamified Rehabilitation