The 8th Annual Retreat-the PhD Program for Translational Medicine

轉譯醫學博士學位學程

第8屆學術成果發表會

Date: September 10th, 2021

Conference link: https://meet.google.com/gau-uwzk-bvg

PIN code：408 164 932#

國防醫學院 National defense medical center

2021轉譯醫學博士學位學程第八屆學術成果發表會議程

時間： 110年9月10日（星期五）

地點： 線上會議 https://meet.google.com/gau-uwzk-bvg

參加對象：本學程七校暨中研院全體教師、學生及行政人員

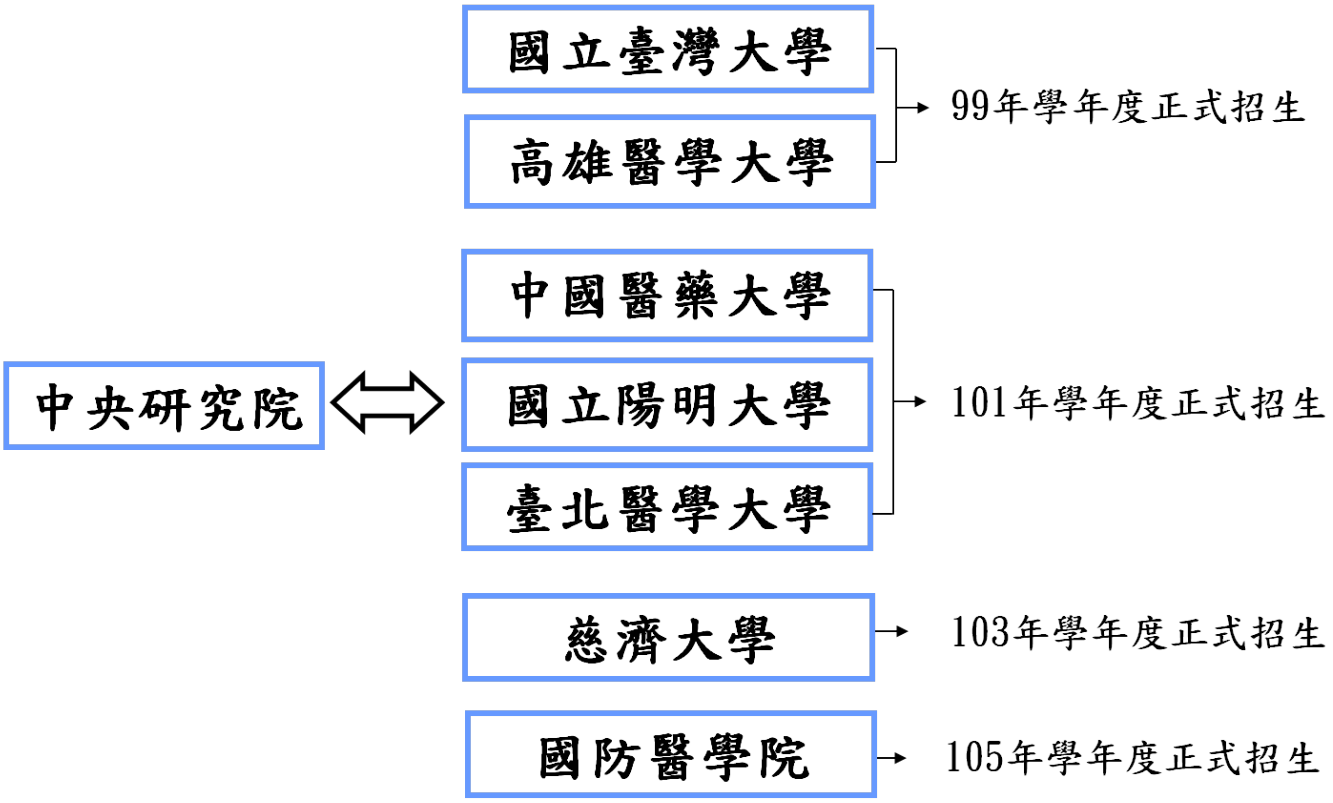
Time	Speaker	Moderator
09:00 - 09:20	Registration (Online)	
09:20 - 09:25	Opening Remarks	國防醫學院 查岱龍 教授/校長
09:25 - 09:55	Keynote speech: Cellular and Molecular Basis of Stem Cell Sheet Transplantation in Cerebral Reconstruction 周中興 副教授 國防醫學院醫學科學研究所	國防醫學院 醫學科學研究所 謝博軒 教授/所長
Oral presentation (I)		
10:00 - 10:20	祁力行（北醫博七）	陳以琳（陽明交通博三）
10:20 - 10:40	陳宛靖（北醫博六）	陳以琳（陽明交通博三）
10:40 - 11:00	王菁（國防博五）	陳以琳（陽明交通博三）
11:00 - 11:20	林威廷（陽明交通博五）	陳以琳（陽明交通博三）
11:20- 11:40	黃菁盈（陽明交通博四）	呂岳峰（臺大博三）
11:40 - 12:00	林柏辰（陽明交通博四）	呂岳峰（臺大博三）
12:00 - 13:30	Lunch	
Oral presentation (II)		
13:30 - 13:50	何偉民（臺大博五）	呂岳峰（臺大博三）
13:50 - 14:10	徐瑜彣（北醫博七）	呂岳峰（臺大博三）
14:10 - 14:30	許芳齊（北醫博七）	梁紹峯（慈濟博三）
14:30 - 14:50	蕭國涵（北醫博四）	梁紹峯（慈濟博三）
14:50 - 15:10	劉昌邑（北醫博四）	梁紹峯（慈濟博三）
15:10 - 15:40	**師生交流座談會 & 頒獎**	謝博軒 教授/所長

備註: 報告人數11人。每位報告15分鐘、提問5分鐘。

Contents

轉譯醫學學位學程簡介

中央研究院與國內七所頂尖大學合作辦理



學程宗旨

合作發展以「轉譯醫學」為核心的生醫研究，以訓練轉譯醫學領域的優秀科學家與醫師群為主軸，從而加強轉譯醫學研究及臨床醫療照護之品質，進一步可以協助企業界提升在全世界生物醫藥之競爭力。

將臨床應用的需求與基礎醫學的研發整合起來的「轉譯醫學」研究，建構於完善的基礎建設之上，包括臨床試驗體系、樣本庫、醫療資訊整合與分析，以及智財、稅法、產學研合作機制之建置。

中研院與合作大學共同扮演教育角色，提供指導、研究資源及相關研究設備等，教學與研究所需要的實驗設備和儀器都可在參與合作的單位裡得到支援。除了培養對於轉譯醫學的研究興趣，並且將這些基礎生命科學的研究發現，轉換到臨床可行的應用層次。

發展方向及重點

- 培育具備競爭優勢的轉譯醫學領域優秀人才
- 重點疾病的研究與開發
- 探索重大疾病的致病因子，作為未來開發預防、篩檢、治療、照護等方案的標的
- 分析具有鑑別性的生物標記(biomarker)，作為未來開發檢驗試劑、藥物等標的；或藉由臨床試驗評估生物標記的可利用性
- 利用基因體學、蛋白體學、生物資訊學等工具或動物模式，研究疾病的致病機制並尋求臨床試驗

學程特色

- 醫師科學家的搖籃：與國內大學醫學院共同培育醫師科學家
- 擁有中研院頂尖師資及豐富教學資源
- 全英語授課
- 學程下設：轉譯醫學組及幹細胞醫學組
- 跨領域前瞻研究主題:
    - 癌症腫瘤研究
    - 心臟血管疾病研究
    - 神經退化疾病研究
    - 幹細胞與再生醫療研究
    - 免疫感染研究
    - 藥物研究與中草藥物開發及新療法
    - 遺傳疾病基因研究
    - 生殖科技研究

Keynote speech

Topic

Cellular and Molecular Basis of Stem Cell Sheet Transplantation in Cerebral Reconstruction

Chung - Hsing Chou M.D., Ph.D. 周中興

Associate Professor, the Graduate Institute of Medical Sciences, National Defense Medical Center

Curriculum Vitae

Chung-Hsing Chou M.D., Ph.D. 周中興
Associate Professor, the Graduate Institute of Medical Sciences, National Defense Medical Center

Current Position

Director of the Division of General Neurology, Department of Neurology, Tri-Service General Hospital

Education

Dr. Chung-Hsing Chou graduated from the School of Medicine, National Defense Medical Center, Taiwan, in 2002 and completed his Neurology Residency at Tri-Service General Hospital, Taipei, in 2008. Dr. Chou earned a PhD in Neuroscience, at Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK, in 2014. Meanwhile, he worked with Professor Michel Modo in tissue regeneration after stroke at the McGowan Institute for Regenerative Medicine, University of Pittsburgh, PA, USA, from 2012 to 2014.

Assignments

He had an oral presentation on "Investigating interactions between human neural stem cells and brain endothelial cells in co-culture systems", in the First UK & Ireland Blood-Brain Barrier Symposium, in Milton Keynes, UK in 2011. In the Military Health System Research Symposium (MHSRS) 2019, he delivered a talk on cerebral reconstruction with the cutting-edge technology by stem cell transplantation in Orlando, FL, USA.

Specialty

Dr. Chou developed a novel model of co-culturing human cerebral microvascular endothelial cells with human neural stem cells for disentangling autocrine, paracrine and juxtacrine signaling between compartments of the neurovascular unit. He came back to Taiwan and applied these techniques to animal models of stroke, traumatic injuries of the brain and spinal nerve. Recently, his invention "The Stem Cell Sheet for Cerebral Neurovascular Reconstruction" was awarded Future Technology Breakthrough Award, in the 2nd Future Technology Exhibition Taiwan 2018 and the 15th National Invention Award Taiwan. He is currently working on several clinical trials based on the preclinical studies of cell therapy for cerebrovascular diseases.

Cellular and Molecular Basis of Stem Cell Sheet Transplantation in Cerebral Reconstruction

Chung-Hsing Chou

OBJECTIVES / BACKGROUND

Traumatic brain injury is one of the leading causes of death and neurological disabilities worldwide. Surgically damaged brain trauma also results in cognitive, sensory, and motor impairments. It is still a challenge to fully restore brain function or promote brain tissue reconstruction.

MATERIAL and METHOD

We first applied human neural progenitor cells (hNPCs) and human cerebral microvascular endothelial cells (hCMECs) to generating the neural progenitor-endothelial cell sheet (NECS) as a novel implant for *in situ* transplantation following surgical brain trauma of adult SD rats to improve neurological function and reduce the lesion size. Genetic analysis for tissue reconstruction and immunological response was done by qPCR. Cellular interactions between different cell types in the NECS were investigated *in vitro* by RNA-seq for either CD31 positive or negative cells sorted by FACS.

RESULT

(1) NECS transplantation improved the neurological function assessed by the modified neurological severity score (mNSS) at days 1, 7, 14, 21 and 28 after cortical resection; (2) qPCR data revealed that NECS reduced mRNA expression of CD86 but enhanced CD209 levels in microglia in the lower chamber of a transwell-separated triple culture model; (3) RNA-seq and GO enrichment analysis on the NECS showed significantly increased expression of genes associated with extracellular matrix, such as *BGN, COL1A1, LOX, LUM* in hNPCs cultured with hCMECs at day 7, in contrast with that of genes associated with cell chemotaxis, such as *CXCL1, CXCL2, CXCL8, EDN2, HBEGF, IL1B, IL6, NR4A1, SAA2* in hCMECs.

DISCUSSION

In this preclinical study, we tried to demonstrate that NECS transplantation promotes cerebral neural plasticity and functional recovery following corticectomy by mediating microglia activation and providing extracellular matrix essential for tissue reconstruction. NPCs and CMECs contribute to brain tissue regeneration via spatially and temporally reciprocal relationships including intercellular and cell-matrix interactions.

Oral presentation

Time	Speaker	Topic
10:00 - 10:20	祁力行 台北醫學大學博七	A Transcriptomic Analysis of Head and Neck Squamous Cell Carcinomas for Prognostic Indications
10:20 - 10:40	陳宛靖 台北醫學大學博六	Rrm2b in the niche modulates stem cell fate in skeletal muscle
10:40 - 11:00	王菁 國防醫學院博五	Post-stroke Delivery of Valproic Acid Promotes Functional Recovery and Differentially Modifies Responses of Peri-Infarct Microglia
11:00 - 11:20	林威廷 國立陽明交通大學博五	miR-X in licensed mesenchymal stem cell exosomes exert immunosuppressive effect through switching macrophages from M1 to M2 phenotype
11:20- 11:40	黃菁盈 國立陽明交通大學博四	CASK Integrates PKR and HCK signal to facilitate Interferon alpha mRNP nuclear export in GM-macrophage during H5N infection
11:40 - 12:00	林柏辰 國立陽明交通大學博四	To investigate the role of meningeal immunity in anti-N-methyl-D-aspartate receptor encephalitis
13:30 - 13:50	何偉民 臺灣大學博五	Comprehensive Analysis of Prognostic Alternative Splicing in High-Grade Gliomas
13:50 - 14:10	徐瑜彣 台北醫學大學博七	Exploring the CLEC18 with potential physiological associations by biobank and TCGA database
14:10 - 14:30	許芳齊 台北醫學大學博七	The serine hydroxymethyltransferase-2 (SHMT2) Initiates Tumorigenesis Development through MYC-Regulated Metabolism in Chemo-resistant Ovarian Cancer
14:30 - 14:50	蕭國涵 台北醫學大學博四	Activation of circulating tumor cell specific cells via hydrogelation of liquid biopsy
14:50 - 15:10	劉昌邑 台北醫學大學博四	Structural investigation of vaccinia virus Entry Fusion Complex

A Transcriptomic Analysis of Head and Neck Squamous Cell Carcinomas for Prognostic Indications

Chi, Li-Hsing (Tex)[1], Wu, TH Alex[1], Hsiao, Michael[2], and Li, Yu-Chuan (Jack)[1]

[1]The Ph.D. Program for Translational Medicine, College of Medical Science and Technology, Taipei Medical University and Academia Sinica

[2]Genomics Research Center, Academia Sinica

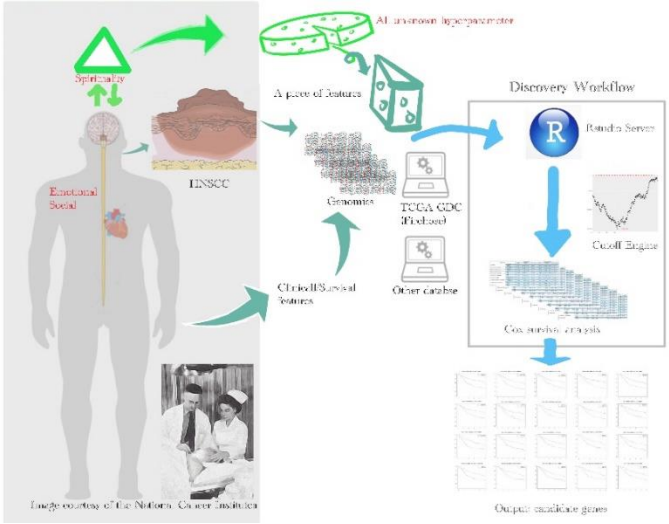
Abstract August 19, 2021

Background: Survival analysis of the Cancer Genome Atlas (TCGA) dataset is a well-known method for discovering gene expression-based prognostic biomarkers of head and neck squamous cell carcinoma (HNSCC). A cutoff point is usually used in survival analysis for patient dichotomization when using continuous gene expression values. There is some optimization software for cutoff determination. However, the software's predetermined cutoffs are usually set at the medians or quantiles of gene expression values. There are also few clinicopathological features available in pre-processed datasets.

Method: We applied an in-house workflow, including data retrieving and pre-processing, feature selection, sliding-window cutoff selection, Kaplan–Meier survival analysis, and Cox proportional hazard modeling for biomarker discovery. In our approach for the TCGA HNSCC cohort, we scanned human protein-coding genes to find optimal cutoff values.

Result: After adjustments with confounders, clinical tumor stage and surgical margin involvement were found to be independent risk factors for prognosis. According to the results tables that show hazard ratios with Bonferroni-adjusted p values under the optimal cutoff, three biomarker candidates, CAMK2N1, CALML5, and FCGBP, are significantly associated with overall survival. We validated this discovery by using the another independent HNSCC dataset (GSE65858).

Conclusion: Thus, we suggest that transcriptomic analysis could help with biomarker discovery. Moreover, the robustness of the biomarkers we identified should be ensured through several additional tests with independent datasets.



K**eywords**: head and neck squamous cell carcinoma (HNSCC); the Cancer Genome Atlas (TCGA); transcriptomic analysis; survival analysis; optimal cutoff; effect size; calcium/calmodulin dependent protein kinase II inhibitor 1 (CAMK2N1); calmodulin like 5 (CALML5); Fc fragment of IgG binding protein (FCGBP); mindfulness meditation

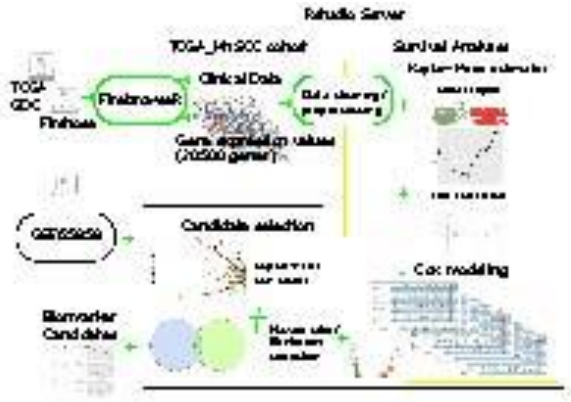


Figure 1: A workflow of HNSCC biomarker discovery. The workflow includes data retrieval from the TCGA GDC data portal, data processing with merging and cleaning, and then performing the survival analyses (within yellow square). The Cutoff engine (in R script: cutofFinder func.HNSCC.R, a serial cutoff for grouping patients with low or high expression of a specific gene, to yield a collection of P values; please see Materials and Methods section for details) might calculate all possible Kaplan–Meier P values (corrected by false discovery rate, FDR, method) to find the optimal cutoff value of gene expression for subsequent Cox modeling. The candidate selection performs (1) dissecting and selection of candidate genes with further Bonferroni adjusted P values and the hazard ratios of a Cox model, based on the results from the survival analyses; (2) survival analyses of the other HNSCC dataset (GSE65858) using Kaplan–Meier estimates (with FDR corrections) and Cox modeling.
The biomarker candidates were consensus results of TCGA and GSE65858. (HNSCC: head and neck squamous cell carcinoma; TCGA: the Cancer Genome Atlas; RNA-Seq: RNA sequencing; GDC: Genomic Data Commons.)

Rrm2b in the niche modulates stem cell fate in skeletal muscle

Wan-Jing Chen[1], I-Hsuan Lin[2], Chien-Wei Lee[3,4], Kiyoshi Yoshioka[5], Yusuke Ono[5], Yu-Ting, Yan[1,6], Yun Yen[2,7,8.9*] and Yi-Fan Chen[1,10,11,12*]

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[8] Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University
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[12] Master Program in Clinical Pharmacogenomics and Pharmacoproteomics, School of Pharmacy, Taipei Medical University

Abstract Skeletal muscle stem cells (satellite cells; MuSCs) found on myofibers (muscle niche) have a major role in muscle growth, homeostasis, and regeneration. The balance among quiescence, differentiation, and self-renewal of MuSCs is tightly regulated by their intrinsic properties and extrinsic signals from the niche. However, how the niche controls MuSC quiescence/differentiation remains unclear. In this study, we found that ribonucleotide reductase M2B (Rrm2b), an essential gene for mitochondrial maintenance, controls MuSC fate in muscle in response to injury. Rrm2b expression is downregulated in the early phase after muscle injury and returns to normal levels in the late phase of regeneration. Rrm2b knockout in mouse myofibers, but not in MuSCs, led to weakness of skeletal muscles, such as a decrease in muscle mass and loss of muscle strength. Loss of Rrm2b in myofibers led to an age-dependent increase in centrally nucleated myofibers. After muscle injury, damaged myofibers were more efficiently repaired in the Rrm2b myofiberspecific knockout mice than the control mice, although these myofibers were thinner and showed decreased functioning. These phenotypes were not observed in the Rrm2b MuSC-specific knockout mice. Rrm2bdeleted myofibers released several myokines, primarily Fgf21, that trigger activated MuSCs to differentiate but not re-enter the quiescent stage to replenish the stem cell pool. This Rrm2b myofiber-specific deletion mouse model could be a potential disease model for mitochondrial myopathy. Overall, Rrm2b in the niche plays a critical role in modulating the quiescence and differentiation of MuSCs, and it may lead to a possible strategy to treat muscle disorders.

Keywords Skeletal muscle; muscle stem cell; regeneration and repair; microenvironment (niche); Rrm2b; FGF21; mitochondrial myopathy.

Post-stroke Delivery of Valproic Acid Promotes Functional Recovery and Differentially Modifies Responses of Peri-Infarct Microglia

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[2] Department of Neurological Surgery, Tri-Service General Hospital, Taipei, Taiwan

[3] Department of Neurological Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

Abstract

The specific role of peri-infarct microglia and the timing of its morphological changes following ischemic stroke are not well understood. Valproic acid (VPA) can protect against ischemic damage and promote recovery. In this study, we first determined whether a single dose of VPA after stroke could decrease infarction area or improve functional recovery. Next, we investigated the number and morphological characteristic of peri-infarct microglia at different time points and elucidated the mechanism of microglial response by VPA treatment. Male Sprague-Dawley rats were subjected to distal middle cerebral artery occlusion (dMCAo) for 90 min, followed by reperfusion. Some received a single injection of VPA (200 mg/kg) 90 min after the induction of ischemia, while vehicle-treated animals underwent the same procedure with physiological saline. Infarction volume was calculated at 48 h after reperfusion, and neurological symptoms were evaluated. VPA didn't significantly reduce infarct volume but did ameliorate neurological deficit at least partially compared with vehicle. Meanwhile, VPA reduced dMCAo-induced elevation of IL-6 at 24 h post-stroke and significantly decreased the number of CD11bpositive microglia within peri-infarct cortex at 7 days. Morphological analysis revealed that VPA therapy leads to higher fractal dimensions, smaller soma size and lower circularity index of CD11b-positive cells within peri-infarct cortex at both 2 and 7 days, suggesting that VPA has core effects on microglial morphology. The modulation of microglia morphology caused by VPA might involve HDAC inhibition-mediated suppression of galectin-3 production. Furthermore, qPCR analysis of CD11b-positive cells at 3 days post-stroke suggested that VPA could partially enhance M2 subset polarization of microglia in peri-infarct cortex. Analysis of VPA-induced changes to gene expressions at 3 days post-stroke implies that these alternations of the biomarkers and microglial responses are implicated in the upregulation of wound healing, collagen trimmer, and extracellular matrix genes within peri-infarct cortex. Our results are the first to show that a low dose of VPA promotes short-term functional recovery but does not alter infarct volume. The decreases in the expression of both IL-6 and galectin-3 might influence the morphological characteristics and transcriptional profiles of microglia and extracellular matrix remodeling, which could contribute to the improved recovery.

Keywords: Distal Middle Cerebral Artery Occlusion (dMCAO), Valproic Acid (VPA), Microglia Activation, Galectin-3 (Gal-3), Ischemic Stroke.

miR-X in licensed mesenchymal stem cell exosomes exert immunosuppressive effect through switching macrophages from M1 to M2 phenotype

Wei-Ting Lin[1,2], Hao-Hsiang Wu[2], Jennifer Hui-Chun Ho[2] , Oscar K. Lee[2]

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[2]Institute of Clinical Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

Abstract

The therapeutic effects of Mesenchymal stem cells (MSCs) are greatly through secreted nano-sized particles, exosomes. MSCs-derived exosomes can manipulate macrophage polarization by inhibiting pro-inflammatory M1 phenotype and promoting anti-inflammatory M2 phenotype through micro RNA (miRNA). The immunosuppressive efficacy of MSCs could be enhanced by licensing them with inflammatory cytokine, which are called "Licensed MSCs (LMSCs)". However, up to now, exosomes from LMSCs that carried specific molecules involved in macrophage plasticity regulation are still inadequately studied. The aim of this study is to investigate the effect of exosomal miRNA from LMSCs in macrophage polarization. We treated MSCs with TNFα and IFNγ (LMSCs) and isolated their exosome to survey the landscape of miRNA, and identify key miR-X enriched in LMSCs. In addition to our macrophage model, primary CD11b$^+$ macrophages (M0) were isolated from tibia and femur in mice, and further differentiated into M1 and M2 macrophages by defining cytokines induction. Treatment with exosomes from licensed MSCs significantly suppressed inflammatory genes and also promoting M2 marker gene expression in mouse macrophage cell. According to our exosomal miRNA sequencing analysis, we discovered miR-X was highly enriched in licensed MSCs-derived exosomes. Furthermore, miR-X lipofection in M1 macrophages could promote them differentiating into M2-like macrophages and inhibiting pro-inflammatory cytokines. In summary, we firstly discovered miR-X play critical role in regulating macrophages polarization, providing us a new sight of therapeutic potential of miR-X in the future.

Keywords: Mesenchymal stem cells, licensed, TNFα, IFNγ, macrophages, M1, M2, polarization, exosomes, miRNAs, miR-X,

CASK Integrates PKR and HCK signal to facilitate Interferon alpha mRNP nuclear export in GM-macrophage during H5N infection

Jing-Ying Huang[1], Shie-Liang Hsieh[1,2]

[1]Doctoral Degree Program of Translational Medicine, National Yang Ming Chiao Tung University and Academia Sinica, Taiwan

[2]Genomics Research Center, Academia Sinica, Taiwan

Abstract

CASK, a calcium/calmodulin-dependent serine threonine kinase, belongs to the MAGUK (membrane-associated guanylate kinase) family and serves as a scaffold protein to mediate protein-binding and signal transduction. Notably, it can also translocate into the nucleus and serve as a transcription co-activator of Tbr-1 to regulate the expression of T-element containing gene during cerebrocortical development. However, the mechanism of CASK nucleus translocation remains unclear, and the function of CASK outside neurons has not been explored yet. Here, we found that CASK translocates from juxta-membrane to nucleus in primary macrophage after incubating with influenza virus (H5N1). This nucleus translocation can be recapitulated by transfecting macrophages with RIG-I or MDA5 ligand, suggesting sensing cytosolic viral RNA by RIG-I or MDA5 is essential for triggering CASK nucleus entry. Next, we screened kinase inhibitors, and found that PKR inhibitor and SRC inhibitor blocks the upregulation of CASK and the nucleus entry of CASK, respectively. HCK is one of the SRC-family kinases (SFK) and is increased transcriptionally in a time-dependent way during H5N1 infection in GM-macrophage. Co-transfection of CASK along with constitutively active HCK triggers CASK nucleus entry in 293T cells. HCK-dependent CASK nucleus entry correlates with the phosphorylation of CASK at serine 395, a site known to be under the control of CDK5. To know the role of CASK in the nucleus, we apply IP-LC-MS/MS and found that CASK interacts with HNRNPs that are involved in mRNA processing and export. In CASK deficient macrophages infected with H5N1, we found that interferon alpha production was selectively dampened by half. Using RNA-FISH, we showed that interferon alpha (Ifna) mRNA nuclear export is impaired and accumulated in the nucleus. These results together established that CASK plays a role in regulating interferon alpha production during H5N1 infection though facilitating Ifna mRNA nuclear export. CASK enters the nucleus in response to cytosolic virus-associated molecular pattern recognition, and this process is regulated by PKR, HCK and CDK5. These findings reveal the mechanism of CASK-mediated post-transcriptional regulation of Ifna in response to H5N1 and sheds light on molecular control of Ifna production during H5N1 infection.

Keywords: CASK, H5N1 influenza A virus, pattern recognition receptor signaling, interactome

To investigate the role of meningeal immunity in anti-N-methyl-D-aspartate receptor encephalitis

Po-Chen Lin

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Department of Neurology, Neurological Institute, Taipei Veterans General Hospital

Abstract:

Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis is an emerging and important antibody-mediated autoimmune encephalitis which causes profound disability and is potentially life-threatening. Tumor, herpes simplex encephalitis, and immune-checkpoint therapy are potential triggers. Although the pathogenicity of anti-NMDA receptor antibody has been demonstrated both *in vivo* and *in vitro* experiments, how and where the autoimmune response evolve are still unknown.

Newly discovered meningeal lymphatics and active immune response on meninges indicate that there are much more communications between central nervous system (CNS) and peripheral immune system than ordinary expectations. Emerging evidences showed that the function of meningeal lymphatics is crucial for the trafficking of macromolecule and immune cells from CNS to the cervical lymph nodes. As the closest peripheral tissue surrounding the brain parenchyma, meninges serve as a “functional niche” for CNS immune surveillance and physiological homeostasis.

In this study, through the herpes simplex encephalitis mouse model and subsequent anti-NMDA receptor encephalitis developed in these mice, we aim to investigate the population and distribution of immune cells and the cytokine expression profiles in the meningeal immunity in different disease status. Furthermore, through manipulating the meningeal lymphatics function, we aim to compare the meningeal immune responses and autoantibody development in the intervention and control groups.

Keywords:

anti-N-methyl-D-aspartate receptor encephalitis, meningeal immunity, meningeal lymphatics, cervical lymph node

Clinical Endpoint Prediction of Differentiating Genes and Transcripts in Pan-Glioma

Wei-Min Ho[1,2], Ta-fu Chen[3], Trees-Juen Chuang[4]

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Abstract

High-grade glioma (HGG) relapses frequently and deteriorates rapidly with a malignant nature. While the synchrony of surgical and irradiating therapy benefits some patients, the treatment effects are palliative and associate with adverse effects. The overall median survival period of HGG is less than one year and the patients suffer from multiple morbidities. Although the survival period is short, we have found a minor portion of HGG patients live longer than the other, and a part of low-grade glioma patients have shorter survival period likewise. This grey area within glioma has stimulated our interest in figuring out the potential genes and isoforms that determine the clinical endpoint of glioma patients. Genetic aberrance, including the status of isocitrate dehydrogenase genes 1 and 2 (IDH1/IDH2), codeletion of chromosome arm 1p and 19q (1p/19q codel), O6-methylguanine-DNA methyltransferase (MGMT) promotor and TERT promoter, have influence on the prognosis of glioma patients. However, there is no substantial evidence determining for how long that glioma patients can survive.

Given that the uncertainty of relapse and the heterogeneity of glioma composition, we would like to investigate the differentiating genes and isoforms affecting the prognosis of HGG patients on the basis of in-silico genomic analysis and to confirm the associating genes and isoforms which complicate glioma evolution.

The ***objective of this proposal*** is to determine the significant genes and isoforms that express in dead glioma patients and the *in vivo* effects of these candidates. The ***central hypothesis*** is that the expression or deficiency of certain genes or isoforms may interfere the progression of glioma. The ***rationale*** underlying this proposal is that proof of the genes or isoforms which affect the survival of glioma patients will help understanding the mechanism behind HGG and will offer a new therapeutic opportunity.

Our ***specific aims*** will test the following hypotheses:

Specific Aim 1. Differential expression analysis for correlation with survival time at both the gene and transcript levels.

Specific Aim 2. Identification of differentially expressed transcript isoforms with the

opposite direction of effect.

Specific Aim 3. Functional analysis of alteration of the identified isoforms in glioma cells.

Specific Aim 4. Identification and validation of the regulatory mechanism of the identified isoforms.

Keyword: Alternative splicing, Bioinformatics, Gene regulation, Glioblastoma Multiforme (GBM), Lower-grade glioma (LGG)

Exploring the CLEC18 with potential physiological associations by biobank and TCGA database

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Abstract

CLEC18 family, consist of CLEC18A, CLEC18B, and CLEC18C, are newly identified C-type lectin. Previous study linked the function of CLEC18 in innate immunity with Toll-like receptors and stimulate the production of cytokines. To investigate the pleiotropic associations of *CLEC18* genes, phenome-wide association study (PheWAS) was applied to survey human phenotypes that are relevant to *CLEC18* genes. We analyzed the genotypic data of 10k Taiwanese subjects with 78 clinical phenotypes. The serum uric acid level significantly associates to the seven variants of *CLEC18A*. In addition to serum uric acid, *CLEC18A* variant also associated with heart rate. Gender stratification analysis indicated the most significantly correlation to *CLEC18* variants was headache/migraine, followed by heart rate, serum uric acid level in the female population. The most associated traits in male population was arthritis, and then the apoplexy. These results identified *CLEC18* relevant phenotypes and implied the pleiotropic effects of *CLEC18* genes in multi-physiological traits.

We also surveyed the *CLEC18* expression profile in TCGA dataset. Upregulated *CLEC18* expressions were observed in Kidney Renal Clear Cell Carcinoma (KIRC) tumors. Pathway enrichment analysis indicated that CLEC18 was associated with angiogenesis pathway and metabolic process. Wild type CLEC18A.Fc had binding ability with specific phospholipids, but when we replaced one substitution at the SCP/CAP domain resulted in the impaired binding lipid function. These observations suggest that CLEC18 may also play a role in irregular lipid metabolisms in KIRC.

The serine hydroxymethyltransferase-2 (SHMT2) Initiates Tumorigenesis

Development through MYC-Regulated Metabolism in Chemo-resistant Ovarian Cancer

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Abstract

c-Myc regulates cell growth, cell proliferation, and differentiation in many cancers and initiates the transcription of many critical genes involved in cell growth by stimulating metabolism. The metastatic and recurrent ovarian cancer results in the major poor prognosis and lethal clinical outcomes due to resistances of chemotherapy. According to previous studies, recurrent high-grade serous ovarian cancer (HGSOC) cells are affected by high expression of c-Myc, and its downstream SHMT2 determines mitochondrial one carbon metabolism. Mitochondrial SHMT2 not only involves in one carbon metabolism but also alters the intermediate purine synthesis, which consequences DNA repair blockade in resistant ovarian cancer cells. To identify the resistance-related genes of MYC-SHMT2-one carbon metabolic axis, we compared the expression profiles between the resistant cell and parental ovarian cancers by tissue microarray and RNA sequence and found that that SHMT2 enhanced in resistant ovarian cancer cells. Based on in vitro siSHMT2 and in vivo metabolomic analysis, SHMT2 inhibitor blocked one carbon metabolic productions and provided a better primary tumor control. In summary, SHMT2 demonstrated a therapeutic potential by altering mitochondrial metabolism in HGSOC cells.

Keywords: c-MYC, The serine hydroxymethyltransferase-2 (SHMT2), metabolism, Ovarian cancer

Activation of circulating tumor cell specific cells via hydrogelation of liquid biopsy

Gwo Harn Shiau 蕭國涵 M.D.

ABSTRACT

Background: Metastasis has been the leading cause of cancer related deaths as in approximately 90% of cancer relevant mortality cases are consequences of the secondary growing tumor at distal organs. Distal metastases are formed by the process of circulating tumor cells (CTCs) leaving the primary tumor and proliferating upon arrival to a new location. Despite the similarity of the cells in respect of sharing the similar variant of antigen presentation, substantial genetic differences between primary tumors and metastases affects clinical care and outcomes such as recurrence and therapeutic resistance. Current tumor antigen identification for cancer treatment is based on the molecular profile of primary tumors. By using current antigen identification parameters stated above, the insight of the heterogeneity within distal cancer cell appears to be lacking which hinders overall effectiveness of T Cell targeting. Moreover, solid tumors do not possess co-stimulatory molecules found on the membrane of antigen presenting cells such as dendritic cells (DCs). Thus, they are unable to initiate T cell activation directly and require DCs to present tumor antigens to T Cell for effective activation. As such, current immunotherapies have shown to be ineffective in long term patient survival. With this regard, we **hypothesize** that tumor cells expressing tumor specific antigen with the addition of costimulatory molecules of DCs will acquire a self-representing DC like entity capable of fully activating T cells by providing both the T-cell-receptor (TCR) signal and associated signals. In addition, CTCs are the primary target of cancer treatment as they cause distal metastasis. If we are successful at activating T cells against the CTC antigen library, we may achieve a protocol to reduce cancer related mortality since metastatic colonization is shown to be the main cause of cancer related deaths. In order of producing a self-representing CTC for T cell stimulation we will firstly utilize a novel technique to increase CTC enrichment yield after liquid biopsy followed by processing the captured CTCs via a novel hydrogelation method for cell membrane engineering.

Objectives: The ultimate goal of this research is to develop novel immunotherapeutic strategies against metastatic cancer to overcome the challenges of immunotherapy caused by the insufficient antigen presentation or costimulation in current cancer treatment models such as CART and DC cell therapy. Specifically, we intend to augment anti-cancer T cell responses by directly using the CTCs vaccine coupled with addition of co-stimulating signal for the metastatic disease. To achieve this goal, three specific aims are proposed:
Specific aim 1: To produce a gelated T cell activator (GTCA) by hydrogelation of CTCs with

anti-CD28 coculturing.
Specific aim 2: To assess the potency of GTCA in T cell activation and cytotoxicity in vitro.
Specific aim 3: To evaluate the feasibility of GTCA use in immunotherapy for metastasis by experimental animal models.

Significance and expected results:

By gelating the CTCs and co-culturing with the needed co-stimulatory signal for T cell activation, we anticipate the presentation of entire antigen constructs among the tumor cell population for T cell activation. Current treatments are relying on predetermined major groups of antigens for targeting cancer cells such as CART cell therapy and APC mediated therapy. This allows minor groups of tumor molecular profile to escape and proliferate onwards resulting in relapse. DCs translating competency of tumor cells are reported to be far from perfect which results in the inability for T cells to eradicate all tumor profiles.
With gelation of CTCs, we hope to produce a comprehensive tumor antigen source covering vast molecular subtypes for T cell activation. We anticipate GTCA to activate tumor antigen specific CD8+ T cells surpassing that of CART and DC vaccine therapeutic coverage together with ease of production and safety of application.

Structural investigation of vaccinia virus Entry Fusion Complex

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Poxviruses are one of the most complicate family in virus realms, unlike most virus that only encode one or two proteins for the host entry. For the cell entry process, this virus family elaborates a huge protein complex that involves at least 10 units. Due to the complexity and no objective structure obtained for this Entry Fusion Complex (EFC), the underlying entry mechanism of poxvirus remains unclear. Based on previous reports, most of entry-fusion proteins are conserved in all poxviruses family and constitute to an EFC. Additionally, the virus-induced fusion could be accomplished both by plasma membrane fusion or endocytosis in different situations. The regulation of these two fusion mechanisms is considered to be achieved by the multifunction of this huge complex. In this study, we focus on the EFC of vaccinia virus, one of well-known the members of *Poxviridae* and is the active vaccine for smallpox. The infection of mature vaccinia virus requires 4 proteins for cell attachment, and 11 protein components of EFC for membrane fusion. Currently, structures of the 4 vaccinia virus envelope protein A26, A27, D8, H3 for attachment were identified. For the 11 components of EFC, only vaccinia virus L1‘s structure had reported in 2008. The structures of other 10 proteins, A16, A21, A28, F9, G3, G9, H2, J2, L5, O3, remain unknown. Here we have utilized the AI system AlphaFold2 to predict each component protein from vaccinia virus EFC. We applied all EFC protein sequence to AlphaFold2 to predict possible 3D structure. The result shows reasonable model for EFC component proteins in single or in couple as a complex sub-unit core. And the output models are further utilized to analysis our protein crystallization data such as G9 recombinant protein. We anticipate the results of this work could extend our understanding of vaccinia virus entry mechanism.

Keywords
Vaccinia virus, entry fusion complex, fusion suppressor, protein crystallography, AI, AlphaFold, Deep Learning.

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