2023

Frontiers in Translational Medicine

DATE **2023.11.24 (FRI)**

Location **Seminar Room**
Institute for Translational Research in Biomedicine
Kaohsiung Chang Gung Memorial Hospital

TRBM 生物醫學轉譯研究所 Institute for Translational Research in Biomedicine
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長庚 高雄

Organized by: Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital

2023 Frontiers in Translational Medicine

Date: Nov. 24, 2023 (Friday)
Time: 09:00 – 16:00
Place: Seminar Room, Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital

Time	Topic	Speaker	Moderator
09:00 – 09:30	Registration		
09:30 – 09:40	Opening Remark	Prof. Samuel HH Chan	
09:40 – 10:10	**Special Lecture** Targeting autophagy as a potential cancer therapy	Chih-Wen Shu, PhD	Prof. Julie YH Chan
10:10 – 10:30	The diagnosis and staging of tau protein disease	Chiung-Chih Chang, MD, PhD	Hong-Tai Tzeng, PhD
10:30 – 10:50	Arsenic-related skin cancers and peripheral vascular disease: From history, biochemistry, immunity, to adverse biological impacts	Chih-Hung Lee, MD, PhD	
10:50 – 11:20	Coffee Break		
11:20 – 11:40	Comparison of long-term clinical outcomes between intracoronary CD34+ cell therapy and optimal medical treatment for end-stage diffuse coronary artery disease	Pei-Hsun Sung, MD	Hong-Tai Tzeng, PhD
11:40 – 12:00	Utilities of lentivirus in studying TRPC6-associated podocytopathies	Chia-An Chou, MD	
12:00 – 13:30	Lunch		
13:30 – 13:50	Loss of Pnn in neurons regulates protein expression of genes involved in frontotemporal dementia and alters locomotor activities in mice	Steve Leu, PhD	Jei-Ming Peng, PhD
13:50 – 14:10	Decipher the role of aryl hydrocarbon receptors in aromatic-uremic toxins induced cognitive disorder	Jenq-Lin Yang, PhD	
14:10 – 14:30	Lipopolysaccharide-induced autophagy increases SOX2-positive astrocytes while decreasing neuronal differentiation in the adult hippocampus	Kay LH Wu, PhD	
14:30– 14:50	Coffee Break		
14:50 – 15:10	Mechanistic investigation in a mouse model of hepatic encephalopathy	Ching-Yi Tsai, PhD	Jei-Ming Peng, PhD
15:10 – 15:30	Calreticulin regulates hepatic stellate cell activation through modulation of calcium signaling.	Kuang-Tzu Huang, PhD	
15:30 – 15:50	Effect of pirfenidone on peritoneal alterations in an uremic mouse model with exposure to peritoneal dialysis fluid	Ding-Wei Chen, PhD	
15:50 – 16:00	Closing Remark	Prof. Julie YH Chan	

Organizer: Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital

Targeting autophagy as a potential cancer therapy

Chih-Wen Shu, PhD

Institute of Biopharmaceutical Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan.

Abstract

Autophagy is a recycling pathway required for normal homeostasis. Dysfunction of autophagy results in different diseases, particularly in cancer. Autophagy is activated to promote cancer malignancy. ATG4B is a dominant protease required for autophagy signaling. We found that ATG4B and its active form are overexpressed in tumor tissues of cancer patients. Knockdown of ATG4B inhibited autophagy, proliferation and mobility of cancer cells, suggesting its oncogenic role in cancer. Moreover, we have screened with FDA approved drug library and found potential drug inhibit ATG4B activity to impede cancer cells. We also design synthetic peptide and RNAi to develop potential modulators for inhibiting cancer cells, hopefully get a starting point for targeted cancer therapy.

The diagnosis and staging of tau protein disease

Chiung-Chih Chang, MD, PhD

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Abstract

神經退化性失智症佔老化疾病的第一位，阿茲海默氏症為最主要的失智症原因。2018 年國家老化研究所-阿茲海默氏症協會(NIA-AA)診斷準則指出，要符合阿茲海默氏症診斷需要同時具備類澱粉蛋白沈積與 Tau 蛋白神經纖維糾結。2023 NIA-AA 準則新增血液指標，早期確診阿茲海默氏症方能引導前瞻性治療。根據 NIA-AA 準則，阿茲海默氏症的診斷由臨床面往前推進到生物標識的層面，治療也針對移除病理性蛋白邁進。此次報告將說明今年申請腦科學研究國科會計畫要完成的研究方法，與近 3 年團隊研究成果，針對阿茲海默症的幾項診斷與指標，分別是非配對誘發電位與沿血管周圍空間 (ALPS) 指數來反映膠狀淋巴功能; 討論血液診斷指標(mAb005, SIOMA, total tau, pTau181, NFL, Abeta 42 and 40) 與生物晶片在診斷與分期早期阿茲海默氏症的可行性, 與針對影像與數位生物特徵資料預測疾病進展。

Arsenic-related skin cancers and peripheral vascular disease: From history, biochemistry, immunity, to adverse biological impacts

Chih-Hung Lee, MD, PhD

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Abstract

砷是自然界地殼中最常見的元素之一。人造的半導體中的砷化鎵及農藥及殺蟲劑中也含有砷。砷在化學元素周期表中，砷與生物細胞內的蛋白質與 DNA 的主要元素氮、磷屬同一族，因此砷在多種生物化學層次上影響著細胞生理與恒定。台灣西南沿海鄉鎮在 1950 年代末期，開始出現流行性皮膚病變、皮膚癌、膀胱癌、烏腳病等。大型的流行病學研究發現這些健康不良效應與飲用深井水有關。其中特別是井水中的砷造成的慢性砷暴露被證明是引起這些不良健康效應的主要原因。砒霜(無機砷為主要成分)的急性中毒與拿破侖、光緒帝的死因有極大關係；有趣的是，砷並不是完全對健康有害的，由於它的調節細胞分化作用，砷在某些類型的急性白血病(免疫細胞白血球的不正常增生)是首選的治療選擇。本演講討論砷對免疫系統的分子調控，與砷對細胞能量來源粒線體的作用機轉，如何來影響細胞分化、生長、微循環的恒定，而造成癌症發生，對化學致癌模式機轉的應用與探討，期望可從病生理機轉的研究來發展對抗癌症的差別化治療。

Comparison of long-term clinical outcomes between intracoronary CD34+ cell therapy and optimal medical treatment for end-stage diffuse coronary artery disease

Pei-Hsun Sung, MD; Hon-Kan Yip, MD

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Abstract

Patients with end-stage diffuse coronary artery disease (EnD-CAD) have multiple and severely diffuse coronary artery stenosis. Vast majority of EnD-CAD patients are unsuitable for coronary intervention due to too small epicardial vessels and poor distal coronary circulation. They usually express intractable angina, present with low exercise capacity, and have high risk for adverse clinical events. Cumulative evidence has shown that application of stem cell therapy (SCT) in the EnD-CAD patients not only reduce angina and dyspnea but also improve left ventricular ejection fraction (LVEF) and functional capacity. However, long-term follow-up data for this kind of high-risk patients undergoing intracoronary (IC) CD34+ cell therapy is still lacking. We sought to compare long-term clinical outcomes between IC CD34+ cell therapy versus optimal medical treatment (OMT) in the EnD-CAD patients. This retrospective analysis were comprised of phase I ((2011-2014, ISRCTN72853206) and phase II (2013-2017, ISRCTN26002902) clinical trials. Stem cell therapy (SCT) group included a total of 68 EnD-CAD patients receiving IC CD34 cell therapy (n=38 in the phase I and n=30 in the II trial). Additionally, OMT group included 30 EnD-CAD patients only treated with guideline-directed medical therapy. All 98 patients in both groups took strict clinical surveillance and received follow-up for at least five years. Data were collected from electronic medical records and telephone contact for judicious scrutiny of clinical events. Clinical symptoms including dyspnea and angina scores, change of LVEF on 3D-echocardiography, and adverse clinical events were compared between the SCT and OMT groups. All data were censored till July 2023. Baseline characteristics did not differ between both groups, with mean age of 65 years and mean LVEF around 54%. More than 70% of patients were male. Eighty-seven percent of EnD-CAD patients had history of surgical and percutaneous coronary intervention, including left main disease in 35% of them. After 5 years of follow-up, a composite of adverse clinical events occurred in more than 65% of the frail EnD-CAD patients of both groups. About one in five EnD-CAD patients in both groups suffered from death from any cause (23.5% vs 20.0%, p=0.797). Majority of death were attributed to cardiovascular death and severe sepsis. Compared with OCT group, SCT group had about two-fold incidences of bailout myocardial revascularization for refractory angina (22.1% vs 10.0%, p=0.171) and major adverse cardiovascular and cerebrovascular events (MACCE, 5.9% vs 3.3%, p=1.000). On the contrary, SCT group had less incidence of hospitalization for heart failure

than OCT group (5.9% vs 10.0%, p=0.673). Among the survivors at the end of follow-up, LVEF was insignificantly higher in the SCT than OMT group (56.2% vs 54.5%, p=0.954). Notably, SCT group had significant improvement of systolic dyssynchrony index than OCT group (5.3 vs 10.0%, p=0.002), which may be related to angiogenic effect of SCT. Kaplan-Meier curve showed the clinical benefits of SCT gradually disappeared about 4 years later. Compared with OMT for EnD-CAD, IC CD34+ cell therapy might slightly improve LV diastolic function, markedly alleviate LV systolic dyssynchrony, and then mildly reduce the risk of hospitalization for heart failure. However, cell therapy is not better than OMT for the reduction of death and MACCE in the long term. We therefore encourage further research to investigate whether second bolus of SCT is necessary for those frail EnD-CAD population.

Utilities of lentivirus in studying TRPC6-associated podocytopathies

Chia-An Chou, MD

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Abstract

Focal segmental glomerulosclerosis (FSGS) is an important cause of chronic kidney disease. Patients suffering from FSGS manifesting nephrotic range proteinuria carry poor renal prognosis, resulting in end-stage renal disease. These FSGS-causing mutations mainly affect the podocyte, an important cell type in the renal glomeruli, to maintain normal glomerular filtration. Currently, at least 60 genes have been identified to cause FSGS. TRPC6 is one of these genes associated with FSGS. The mutation in TRPC6 causing FSGS can be classified into gain-of-function, loss-of-function, and unchanged-function mutations. The function is defined by the amplitude of calcium influx induced by stimulation compared with the wild-type gene. The lentivirus is an ideal tool for transducing gene-of-interest into dividing and non-dividing cells to establish stable expression cell lines. It can overexpress or silence the gene of interest in cells for experiments. Using the lentivirus, the genetically encoded calcium indicator gene is transduced into podocytes as a tool to measure the calcium influx in podocytes. Regulators known to interact with TRPC6 were tested in the podocytes to understand the effect of TRPC6 channel function on the podocytes. In summary, combining the lentivirus and the genetically encoded calcium indicator is useful for studying the calcium channel function.

Loss of Pnn in neurons regulates protein expression of genes involved in frontotemporal dementia and alters locomotor activities in mice

Steve Leu, PhD

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Abstract

The regulation of pre-mRNA alternative splicing in neurons is considered to be associated with neurodegeneration disease. In the present study, we applied an inducible neuron-specific gene depletion mouse model to determine whether the expression of Pnn, a mRNA splicing regulator, is involved in the induction of neurodegeneration. Six-weeks old male mice carrying CaMKII-CreERT2 gene cassette and $Pnn^{flox/flox}$ allele were injected with tamoxifen to induce the disruption of Pnn gene structure. In addition to histopathological and biochemical examination, brain MRI analysis and the observation on behavior alteration were also performed on mice with neuronal Pnn depletion. Locomotor activity measurement showed that the travel distance, ratio of moving/resting, central time, and central distance in neuronal Pnn-depleted mice were all higher than that in wild type mice since three months after induction of Pnn gene disruption. Moreover, the decrease in gripping force was observed in mice with neuronal Pnn depletion. MRI analysis further demonstrated that loss of Pnn in neurons leads to cerebral ventricular dilatation and hippocampal atrophy in 10-month-old mice. Mass spectrometry-based immuno-precipitation proteomics indicated the interaction between Pnn and frontotemporal dementia (FTD)-associated proteins, including TDP-43, FUS, and hRNPA1. It is worth noting that both immunofluorescent stainings and Western blottings demonstrated the up-regulation of FTD-associated proteins in hippocampus and cerebral cortex of mice with neuronal Pnn depletion. However, the transcriptional level of genes involved in FTD were not altered by Pnn depletion. In conclusion, loss of Pnn in neurons leads to behavioral alteration in mice with regulating protein expression of genes involved in FTD, implying the involvement of Pnn deficiency in neurodegeneration.

Decipher the Role of Aryl Hydrocarbon Receptors in Aromatic-Uremic Toxins Induced Cognitive Disorder

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Abstract

Chronic kidney disease (CKD) is with a characterization of progressing loss of renal function; commonly, CKD progression leads to multiple comorbidities, cognitive dysfunction is one of them. Our previous study reported that accumulation of uremic toxins, especially indoxyl sulfate (IS) and p-cresol sulfate (PCS), could penetrate into brain tissue and induce neuronal inflammation and contribute to CKD-triggered cognitive impairment. The current study aims to understand how IS and PCS passing through endothelial cells and affecting endothelial physiology, consequently, compromises cerebral-endothelial and blood-brain-barrier (BBB) function. The results of Western blotting assay indicated expression of inflammatory cytokines, caspase-1, IL-1β, and IL-18, and tight-junction proteins were altered in IS/PCS-treated cerebral endothelial cells. In the meantime, immunocytochemistry images showed that the aryl hydrocarbon receptor (AhR) was activated and relocated to nuclei and the organic anion transporter (OAT) inhibiter, chloroquine, prohibited IS-induced AhR nuclear relocation of endothelial cells. The mitochondrial function was examined by Agilent Seahorse XF analyzer, which indicated treatment of IS and PCS compromised ATP production of mitochondria in cerebral-endothelial cells respectively. Moreover, the ChIP-seq assay was performed to determine genes that are regulated by AhR. The analytical results of ChIP-seq suggested AhR-regulated genes involving in function of cell junction, cell adhesion, integral component of membrane, organelle envelope, cytoplasmic vesicle, intracellular transport, and G-protein signaling. Therefore, we postulated that IS and PCS pass through endothelial membrane via certain types of OATs/OATPs and activate AhRs relocating to nuclei, subsequently, induce endothelial inflammation, alter expression of tight-junction proteins, and dysregulate permeability of BBB. The goal of our study is to decipher mechanisms of aromatic uremic toxins, specially IS and PCS, induced cognitive impairment and find out possible treatments via blocking IS/PCS endothelial transcytosis, suppressing endothelial AhR activity, preventing endothelial dysfunction, and maintaining BBB permeability.

Lipopolysaccharide-induced autophagy increases SOX2-positive astrocytes while decreasing neuronal differentiation in the adult hippocampus

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Abstract

Inflammation alters neural stem cell (NSC) lineage from neuronal to astrogliogenesis. However, the underlying mechanism is elusive. Autophagy contributes to the decline of adult hippocampal neurogenesis under the *E. coli* lipopolysaccharide (LPS) stimulation. SRY-Box transcription factor 2 (SOX2) is critical for the NSCs self-renewal and proliferation. Contradictory, SOX2 facilitates autophagy by suppressing the mammalian target of rapamycin (mTOR). In this study, LPS (5 ng • $100g^{-1}$ • $hour^{-1}$ for 7 days) was peritoneal infused to male Spray-Dawley rats (8 weeks old) to induce a mild systemic inflammation. Beclin 1 and the autophagy protein 12 (Atg12) were significantly upregulated accompanied by SOX2 increment while the Ki67- and doublecortin (DCX)-positive cells in the dentate gyrus were decreased. The fluorescent micrographs indicated that the co-localization of Beclin 1 and SOX2 was increased in the subgranular zone (SGZ) of the dentate gyrus. Enhanced SOX2 was largely distributed in the astrocytes instead of neurons. Synchronically, the levels of phospho(p)-mTOR, p-mTOR/mTOR ratio, p-P85s6k, and p-P85s6k/P85s6k ratio were suppressed. 3-Methyladenine (an autophagy inhibitor) intracerebroventricular infusion effectively prevented the increases of SOX2, Beclin 1 and Atg12. The SOX2-Baclin colocalized cells and the SOX2-GFAP colocalized cells were reduced. The levels of p-mTOR and p-P85s6k were enhanced. Most importantly, the number of DCX-positive cells was preserved. Together, this study suggested that autophagy-reduced neural differentiation under LPS might be a result of a shift of NSC lineage from neurogenesis to astrogliogenesis while SOX2-associated inactivation of mTOR/P85s6k might be the crux of the switch.

Mechanistic investigation in a mouse model of hepatic encephalopathy

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Abstract

Acute liver failure is a devastating consequence of hepatotoxic liver injury that can lead to the disruptions of brain functions called hepatic encephalopathy (HE). Our previous work demonstrated that neuronal death in the rostral ventrolateral medulla (RVLM), a key neural substrate that maintains blood pressure and sympathetic vasomotor tone, leading to baroreflex dysregulation, is causally related to death in a mouse model of HE. Based on transcripts analysis of astrocyte purified from mouse brainstem during experimental HE, the gene expression of lipocalin-2 (Lcn2) was significant increase. Therefore, the present study was to delineate the role of astrocyte-secreted Lcn2 during HE. We found that i.c.v. infusion of Lcn2-neutralizing antibody into C57BL/6 mice prolongs survival and reduces apoptotic cell death in RVLM and reverses defunct sympathetic vasomotor tone during HE. Furthermore, gain-of-function and knockdown experiments in primary neuron cultures demonstrated that Lcn2 acts through Lcn2 receptor (Lcn2R) to exert neurotoxic effects and that this neurotoxic action is exerted via upregulating pyruvate dehydrogenase kinase (PDK) 1 and PDK3 through MAPK/ERK pathway, thereby inhibiting pyruvate dehydrogenase (PDH) activity via PDH phosphorylation and reducing mitochondrial ATP production. In conclusion, we demonstrated that Lcn2/Lcn2R signaling induces neuronal death by interfering ATP production in neurons, which leads to the high fatality associated with HE in acute liver failure. Mechanistically, we found that Lcn2 upregulates PDK through the MAPK/ERK pathway, thereby reducing PDH activity and mitoATP production.

Calreticulin regulates hepatic stellate cell activation through modulation of calcium signaling.

Kuang-Tzu Huang, PhD

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Abstract

Liver fibrosis is characterized by excessive deposition of extracellular matrix (ECM) in the liver as a wound healing process, which is triggered by various etiologies including viral infection, alcohol consumption, dysregulation of autoimmunity, and metabolic imbalance. If not managed properly, liver fibrosis can progress to cirrhosis, ultimately leading to portal hypertension and hepatic failure. The initiation of liver fibrosis largely depends upon the extent of chronic injury, which stimulates the production of profibrotic factors from damaged hepatocytes and infiltrating macrophages, resulting in activation of hepatic stellate cells (HpSCs). Activated HpSCs are the major producer of the ECM and play a central role in liver fibrogenesis. It has been widely accepted that liver fibrosis is a dynamic and reversible process. Elimination of activated HpSCs or reversion to a quiescent state can be a feasible strategy for resolving the disease. Currently, there are no approved medications that can effectively reverse liver fibrosis, further highlighting the urgent need for novel therapeutic targets. Calreticulin (CRT) is a molecular chaperone that normally resides in the lumen of the endoplasmic reticulum (ER), important in protein folding and trafficking through the secretory pathway. CRT also plays a critical role in calcium (Ca^{2+}) homeostasis, with its Ca^{2+} storage capacity and ability to control Ca^{2+} mobilization. In the current study, we aimed to demonstrate its function in directing HpSC activation. In a mouse liver injury model, CRT was up-regulated in HpSCs in response to the fibrogenic stimuli. In cellular experiments, we further showed that this activation was through modulating the canonical TGF-β signaling. Knocking down CRT in HpSCs abrogated TGF-β-induced Smad2/3 phosphorylation but had no effect on non-canonical protein kinases. As down-regulation of CRT in HpSCs elevated intracellular Ca^{2+} levels through a specific term of Ca^{2+} influx, named store-operated Ca^{2+} entry (SOCE), we next examined whether moderating SOCE affected TGF-β signaling. Interestingly, blocking SOCE with a specific inhibitor BTP-2 or knocking down the Ca^{2+} channel Orai1/Orai2 had little effect TGF-β-induced gene expression. In contrast, inhibition of Ca^{2+} release from the ER by using the inositol trisphosphate receptor (IP3R) inhibitor 2-APB increased TGF-β signaling. Treatment with 2-APB did not alter SOCE but decreased intracellular Ca^{2+} at the basal level. Indeed, adjusting Ca^{2+} concentrations by EGTA or BAPTA-AM chelation further enhanced TGF-β-induced gene expression. Taken together, our results suggest a crucial role of CRT in the liver fibrogenic process through modulating Ca^{2+} mobilization and TGF-β signaling in HpSCs. This study may provide new information and help advance the current discoveries for liver fibrosis and its complications.

Effect of pirfenidone on peritoneal alterations in an uremic mouse model with exposure to peritoneal dialysis fluid

Ding-Wei Chen, PhD

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Abstract

Peritoneal dialysis (PD) is restricted by fibrotic remodeling of the peritoneal wall, a transforming growth factor-beta (TGFB1)-mediated process resulting from long-term exposure to high glucose in most PD fluid that eventually cause peritoneal alterations in PD patients. Pirfenidone is a potent antifibrotic agent approved for treating idiopathic pulmonary fibrosis. However, its impact on peritoneal fibrosis has not been explored. We achieved a more clinically relevant mouse model of peritoneal failure by inducing uremia through 5/6 nephrectomy under peritoneal dialysis exposure conditions. The results revealed that daily oral administration of pirfenidone inhibited peritoneal thickness, fibrosis, levels of inflammatory cytokines, and markers of epithelial-mesenchymal transition in the mouse peritoneum exposed to peritoneal dialysis fluid. Our study also revealed that the Vitamin D receptor may be crucial in facilitating the protective effects of pirfenidone against peritoneal failure.