POSTGRADUATE RESEARCH DAY

2020
5th-6th Nov

School of Biomedical Sciences

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Lo Kwee Seong Integrated Biomedical Sciences Building
Area 39, The Chinese University of Hong Kong

The Executive Committee of the 8th Postgraduate Student Association of the School of Biomedical Sciences

SBS Postgraduate Research Day 2020

Faculty of Medicine
The Chinese University of Hong Kong

Welcome Message from the Director of School of Biomedical Sciences

I would like to extend a very warm welcome to all students, teachers and guests to the *11th SBS Postgraduate Research Day*. Since its inception in 2010, this student-organized event has been an annual flagship programme of our School in the past ten years. I am looking forward to hearing from our postgraduate students their exciting research results based on their hard work and the guidance provided by their thesis supervisors.

This year's SBS Postgraduate Research Day is very special as it coincides with the 10th year anniversary of the School of Biomedical Sciences. Our MPhil-PhD programme has trained over 300 postgraduate students in the past 10 years with many of them have gone on to be professors, researchers, or other professionals in medical, biomedical and pharmaceutical fields. Postgraduate students are a critical component of our research enterprise and the School has spared no efforts in providing high quality scientific training and state-of-the art research infrastructures in the past 10 years. In the coming years, we will continue to recruit top talents and further upgrading our core facilities with cutting edge technology so that School members can pursue their innovative scientific ideas.

The SBS Postgraduate Research Day is a forum for our students to look beyond their own research topics and be curious about the research conducted by their fellow students. The past year has been challenging for most of us. Although this year's Postgraduate Research Day will be conducted on a virtual meeting platform, I am very encouraged by the enthusiasm from our students and faculties. I hope this meeting platform can further enhance collaborations and sharing of ideas among different laboratories.

I would like to take this opportunity to thank members of the organizing committees, especially Ms. Hao Suyu and Zhu Yu of the SBS Postgraduate Student Association; all the faculty members who have provided invaluable advice to their students; and last but not least, members of the Graduate Education Office headed by Prof. Woody Chan and Prof. Zhao Hui, for their superb administrative support and guidance to this event.

On behalf of all members of the School of Biomedical Sciences, I wish you all a very successful Postgraduate Research Day 2020.

Andrew M. Chan, PhD
Professor and Director,
School of Biomedical Sciences
Oct 2020

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Rundown of Postgraduate Research Day 2020
School of Biomedical Sciences (SBS)

November 5th (Thursday – Day 1)		
08:45	**Entry Via ZOOM link opens**	
09:00–09:35	***Opening Ceremony***	
09:00–09:05	*Introduction (special thanks to Honorable Guests and Sponsors)*	
09:05–09:15	*Opening remarks by Professor Rocky S. Tuan, Vice-Chancellor*	
09:15–09:25	*Opening remarks by Professor Wai Yee Chan, Pro-Vice-Chancellor*	
09:25–09:35	*Welcome Speech by Professor Andrew M. Chan, School Director*	
09:35–10:55	**Rm1: Powerpoint Posters Presentation CBET 01-11 (session 1)**	**Rm2: Powerpoint Posters Presentation DRB 01-11 (session 1)**
	Break (10 minutes)	
11:05–12:25	**Rm1: Powerpoint Posters Presentation CBET 12-18 (session2)**	**Rm2: Powerpoint Posters Presentation DRB 12-22 (session 2)**
	Lunch	
14:00–15:30	**Rm3: Powerpoint Posters Presentation NVMB 01-12 (session 1)**	**Rm2: Powerpoint Posters Presentation DRB 23-34 (session 3)**
November 6th (Friday – Day 2)		
08:50–09:00	**Entry Via ZOOM link opens**	
09:00	*Brief introduction: rules of oral presentation*	
09:05–10:25	**Oral Presentation No. 1-4**	
	Break (5 minutes)	
10:30–11:30	**Oral Presentation No. 5-7**	
	Break (5 minutes)	
11:35–12:35	**Oral Presentation No. 8-10**	
	Break (15 minutes)	
12:50–13:00	***Prize Presentation Ceremony***	

Table of Contents

Cancer Biology and Experimental Therapeutics Theme 5

Developmental and Regenerative Biology Theme 28

Neural, Vascular, and Metabolic Biology Theme 65

CANCER BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Cancer Biology and Experimental Therapeutics

Title	Author	Abstract Number
Computational investigation of liver cancer immunotherapy resistance based on single-cell transcriptomics	CAO Jianquan	CBET1
Immune Infiltration Analysis of RAC1 mutations in Head and Neck Cancers	CHAN Hoi Yin	CBET2
Nuclear receptor TLX promotes cancer stemness and epithelial-mesenchymal transition (EMT) in prostate cancer via its direct transcriptional regulation of CD44	CHOW SinTing	CBET3
Stromal leukotriene-induced neutrophil infiltration drives tumor progression by enhancing stemness of cancer cells	CHU Chun	CBET4
Altered Disease-Associated Human TCR Repertoire and CDR3 Motif Analyses Depict Distinct TCRβ Profile in SLE	FUNG Sin Hang	CBET5
Investigation of the role of GSH conjugation in the formation of urinary pyrrole-amino acids adducts	HE Xin	CBET6
A novel analytical technique to assess intestinal motility in zebrafish larvae	HUI Chung Man Jessica	CBET7
Enhancer dysregulation of myeloid-derived suppressor cells in hepatocellular carcinoma	LAW Wingyan	CBET8
Integrated analysis of differential expressed genes and somatic variants in transcriptomic data of prostate cancer patients reveals potential diagnostic and prognostic markers	LIANG Yonghao	CBET9
Investigation of PA-induced DNA damage and repair response in HepaRG cells and rat livers	LONG Yun	CBET10
Investigation of pyrrolizidine alkaloid-induced liver injury in zebrafish	PAN Yueyang	CBET11
Characterization of novel allergens in storage mites	SHIN Soo Kyung	CBET12
Resistance to immune checkpoint targeting in hepatocellular carcinoma: Role of tumor interferon signaling	TU Yalin	CBET13
Investigating the HDAC8-mediated 3D chromatin conformation in T cells: role in cancer immunotherapy	WANG Jing	CBET14
Transcriptome immune signatures of ALK-altered HNSCC	WANG Lan	CBET15
Effect of diet intake on the gut microbiome composition of allergic patients and dynamic structural process of N-cycle functional modules.	WANG Yiwei Evy	CBET16
The causal inference among hypertension, antihypertensive drugs and osteoporosis Using Mendelian randomization study	XIANG Yong	CBET17
Integrating Clinical Data and Imputed Transcriptome from GWAS to Uncover Complex Disease Subtypes: Applications in Psychiatry and Cardiology	YIN Liangying	CBET18

CBET1

Computational investigation of liver cancer immunotherapy resistance based on single-cell transcriptomics

Jianquan Cao, Zhewen Xiong, Jingying Zhou, Stephen Chan, Kevin Yip, Alfred Cheng

Supervisor: Alfred Cheng

Liver cancer is one of the most prevalent cancer types and has the fastest increase in mortality over the past decade. However, most patients do not respond to recent immune checkpoint therapies and our understanding of the underlying resistance mechanisms is limited. Hepatocellular carcinoma (HCC) is the most common type of liver cancer and most of the HCCs in the Chinese population can be attributed to the hepatitis B virus (HBV) infection. Here, we investigated resistance mechanisms by profiling single-cell transcriptomics of biopsies from advanced HBV-related HCC patients undergoing anti-programmed death 1 (PD1) immunotherapy. Using Chromium single cell 3' RNA sequencing, we revealed the complexity of the tumor microenvironment in HCC patients. We further characterized features of HCC microenvironment that correlates with responses to anti-PD1 treatment. Our analyses will facilitate identification of potential responders and future improvement of therapeutic efficacy.

CBET2

Immune Infiltration Analysis of RAC1 mutations in Head and Neck Cancers

Helen Chan Hoi Yin, Hoi-Lam Ngan, Vivian Wai Yan Lui

Supervisor: Vivian Wai Yan Lui

RAC1 is a known oncogene which is found to be essential for invasion, EMT, intravasation and metastasis in different cancer type. Extensive research has been carried out in melanoma focusing on the p.P29S mutations, though it remains undruggable till now. Comprehensive pan-cancer analyses first reveal *RAC1* p.A159V hotspot mutation, followed by several G-box *RAC1* mutations (C18X, K116X and G15S), which appear in 2.94% (15/510) head and neck squamous cell carcinoma (HNSCC) patients associated with significant poorer disease-free and overall survival ($P<0.01$).

While HNSCC is one of the most highly immune infiltrated cancer types with promising results in clinical trials of immune checkpoint inhibitors, e.g. nivolumab, we question on whether immune-related events contribute to the dismal outcomes of *RAC1*-mutated HNSCC patients. From the TIMER and CIBERSORTx analyses, we found that *RAC1* p.A159V is associated with an immunosuppressive immune microenvironment with elevated neutrophil and M2 macrophage infiltration. Gene set enrichment analysis of *RAC1*-mutated HNSCC also demonstrated increased interleukin-6 production, a pro-tumorigenic inflammatory cytokine known to be involved in tumor progression as well as an essential signal for M2 macrophage polarization. This elevated immune infiltration is not shown in p.P29S in melanoma, indicating a specific role of RAC1 GTPase activity in the tumor immune microenvironment in HNSCC. Animal models will be used subsequently to further validate the findings.

This will be the first study highlighting the clinical relevance of RAC1 mutations in HNSCC and revealing the immunological roles of *RAC1* aberrations. These findings suggest a potentially important role of RAC1 aberrations in HNSCC progression, which may serve as prognostic biomarkers and potential drug targets for HNSCC.

CBET3

Nuclear receptor TLX promotes cancer stemness and epithelial-mesenchymal transition (EMT) in prostate cancer via its direct transcriptional regulation of CD44

Sin Ting CHOW, Youjia LI, Dinglan WU, Franky Leung CHAN

Supervisor: Prof. CHAN Leung Franky

Treatment of prostate cancer with existing therapies inevitably results in tumor replase and development of aggressive, androgen-independent disease. Studies over the past decade suggest the presence of a small subset of cancer stem-like cells, which attributes the aggressiveness of prostate cancer. NR2E1 or TLX (Tailless), has been known to maintain stemness and self-renewal ability of neural stem cells. It is overexpressed in prostate cancer and raises with high Gleason-scored and hormone-refractory tumor. It is up-regulated with cancer stem cell markers (e.g. CD44 and CD133) in prostatospheres and VCaP-CRPC xenografts. Base on these, we hypothesize that TLX may promote cancer stemness and EMT in prostate cancer. TLX was overexpressed in LNCaP and PC3 cells which have low endogenous TLX levels, and knockout in 22Rv1 and DU145 cells which have high endogenous TLX levels. We demonstrated that in prostate cancer cells, TLX promoted colony formation, sphere formation and migration capacities. It positively regulated mRNA levels of CD44 and EpCAM. In hormone refractory DU145 and PC3 cells, TLX enhanced invasiveness and in *vivo* tumorigenicity and metastasis. Luciferase reporter assay and ChIP showed the binding of TLX to the promoter of CD44. These suggested that TLX may promote cancer stemness and EMT in prostate cancer by transactivating CD44, and it may act as a hub between these two properties.

CBET4

Stromal leukotriene-induced neutrophil infiltration drives tumor progression by enhancing stemness of cancer cells

Chun Chu[1], Wen-Zhen Lin[1], Ka-Long Chu[1], Wing-Tai Cheung[1]

Supervisor: Wing-Tai Cheung

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, HKSAR, China

A large body of clinical evidence suggests that neutrophil infiltration correlates with a poor outcome in cancer patients. However, whether tumor-associated neutrophils (TANs) directly contribute to disease progression as well as the action mechanism still remains obscure. Using a mouse xenograft model of *Mas*-overexpressing CHO cells (Mc0M80)-derived experimental ovarian cancer, gene expression profiling, proteomics analysis, immunotyping and histological examination were performed. In addition to infiltration of inflammatory cells, high expressions of *Gro* chemokines and coactosin-like protein (COTL1) were detected in xenograft. Consistent with previous reports that COTL1 enhanced leukotriene (LT) production, a significant amount of leukotriene B4 (LTB4) was detected in xenograft. Subsequently, we found Ly6G^{+} neutrophils and ER-TR7^{+} cancer associated fibroblasts (CAFs) contributed LTB4 synthesis in the stroma of xenograft. Of interest, pharmacological inhibition of arachidonate 5-lipoxygenase (ALOX5) suppressed xenograft growth *in vivo*. LTB4 did not exert any growth effect on the Mc0M80 cells, isolated CAFs and peritoneal neutrophils *in vitro*, except acting as a potent chemoattractant for neutrophils. Depletion of neutrophils limited tumor growth *in vivo*. Furthermore, tumor size of xenograft was positively correlated with the blood Ly6G^{+} neutrophil number. Co-culture with peritoneal neutrophils promoted Mc0M80 cell growth and enhanced the expression of *Mas, Cxcl1, Cxcl3*. In addition, a panel of stemness markers (*Cd44, Sox2, Cxcr4 and aldehyde dehydrogenase 1a1*) were upregulated in Mc0M80 cells that co-cultured with peritoneal neutrophils. The presence of cancer stem cells in xenograft was confirmed by xenograft re-transplantation. Consistent with a key role of LT in carcinogenesis, bioinformatics analysis of TCGA database indicated an upregulation of ALOX5 in several devasting cancers, such as cholangiocarcinoma and pancreatic carcinoma. Taken together, neutrophil infiltration orchestrated a LTB4-mediated positive feedback loop of neutrophil-promoted carcinogenesis. Importantly, this study unveils ALOX5 being a novel therapeutic target for cancers that have no curable treatment available.

CBET5

Altered Disease-Associated Human TCR Repertoire and CDR3 Motif Analyses Depict Distinct TCRβ Profile in SLE

Sin-Hang FUNG, Kai-Kei MIU, Stephen Kwok-Wing TSUI

Supervisor: Stephen Kwok-Wing TSUI

T lymphocyte subsets are critically involved in the development of various autoimmune diseases, includes systemic lupus erythematosus (SLE). T-cell Receptor (TCR) repertoire sequencing is increasingly used to determine the pathological contexts, such as autoimmune disease, infection, and cancer. Upon specific exposure to antigen, some T-cells clones are expanded and activated. As lymphocytes are continuously produced and circulated in the bloodstream for antigens recognition, the TCR repertoire landscape is extremely dynamic. In SLE, systemic inflammation and organ damage is usually caused by T-cell infiltration. The diversity of the TCR repertoires can be used to determine an individual's immune profile and history and served as a biomarker. In TCR, the complementarity-determining region 3 (CDR3) region is often referred to as the hypervariable region, which is responsible for antigen recognition. In this study, we have applied algorithms to characterize the circulating TCRβ repertoire landscape and CDR3 motif in the SLE context from bulk RNA transcript. Distinct TCRβ repertoire profile and CDR3 motif in SLE are observed.

CBET6

Investigation of the role of GSH conjugation in the formation of urinary pyrrole-amino acids adducts

Xin HE, Lin ZHU, Ge LIN

Supervisor: Ge LIN

Pyrrolizidine alkaloids (PAs) are extensively distributed in a variety of plant species, plenty of which are easily accessible for human beings in the forms of herbal medicines and dietary supplements. PAs are generally classified into three types, namely retronecine-type PA, otonecine-type PA and platynecine-type PA, the former two types of PAs undergo metabolic activation mediated by hepatic P450 and subsequently generate the reactive metabolites called dehydro-pyrrolizidine alkaloids (DHPAs), which could bind with cellular proteins to form pyrrole-protein adducts (PPAs), leading to hepatotoxicity or conjugate with the reduced form of glutathione (GSH) to generate pyrrole-GSH adducts. Apart from binding with cellular proteins and GSH, DHPAs can react directly with cellular amino acids to form pyrrole-amino acid adducts (PAAAs), which can also be generated from the degradation of PPAs and pyrrole-GSH adducts. All PAAAs are eventually excreted from urine. In our previous study, four PAAAs, namely pyrrole-7-cysteine, pyrrole-9-cysteine, pyrrole-9-histidine and pyrrole-7-acetylcysteine, were detected from the urine of PA-treated SD rats with long persistence, and thus considered as potential urinary biomarkers for diagnosis of PA-induced liver injury in drug-induced/herb-induced liver injury (DILI/HILI) patients. GSH, as an important antioxidant for many biochemical functions, competitively reacts with DHPAs against cellular proteins after PA exposure, eventually leading to GSH depletion as well as formation of pyrrole-GSH adducts, which would undergo mercapturic acid degradation followed by urine excretion and thus regarded as a detoxification pathway.

Hence, in the present study, we firstly investigated the role of GSH conjugation in the formation of urinary PAAAs. Male Sprague Dawley rats were randomly divided into two groups (5/group), specifically RTS and BSO+RTS group. A GSH synthesis inhibitor, buthionine sulphoximine (BSO), was administrated to rats of BSO+RTS group via drinking water in the concentration of 20 mM with free access, whereas the drinking water of RTS group was only tap water. Retrorsine (RTS), a representative toxic PA, was chosen and orally administered with a single dose at 5 mg/kg to rats of both groups. Urine samples were collected and analyzed for the aforementioned four PAAAs by LC/MS. The results showed that BSO treatment significantly reduced the daily excretions of pyrrole-7-cysteine and pyrrole-7-acetylcysteine, indicating that these two PAAAs were mostly derived from pyrrole-GSH adducts. In addition, pyrrole-9-cysteine was slightly decreased by BSO treatment, implying that pyrrole-GSH adducts contribute to a less extent of its formation. However, BSO treatment did not significantly affect the daily excretion of pyrrole-9-histidine. Our result also indicated that the cysteine in GSH might have a significantly higher affinity to interact at position 7 than position 9 of DHPAs.

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CBET7

A novel analytical technique to assess intestinal motility in zebrafish larvae

HUI Chung Man Jessica, J.A. Rudd

Supervisor: J.A. Rudd

Motility disorders of the gastrointestinal (GI) tract can occur due to a variety of reasons and can be debilitating. Chronic constipation or diarrhoea may occur following alteration of mechanisms in the GI tract itself, or may be secondary to other diseases, such as diabetes or cancer. Most preclinical studies on motility using higher order animals (e.g. mouse, rat) have been performed using invasive and/or indirect techniques since the thick layers of skin and muscle preclude a direct visualisation of the GI tract. The zebrafish (*Danio rerio*) is being used increasingly in biomedical research due to their many advantages such as small size, easy maintenance, and transparency. In early developmental stages, zebrafish larvae have an organised intestinal tract, that are divided into three segments (anterior, mid- and posterior regions) and is easily visualised providing an advantage when studying motility mechanisms in vivo. Some previous studies have studied GI tract motility in zebrafish, but the techniques employed are not robust and mainly use manual approaches. For example, animals are fed and the investigator tracks the distance travelled by measuring the transit time of the food bolus over specific distances, or by recording the duration required for the bolus to be excreted. This only indirectly measures velocity and contraction/relaxation parameters are not normally obtained. In the present studies, therefore, we aim to introduce a novel and advanced analytical technique that can assay intestinal motility in more detail. Zebrafish larvae at 5, 6 and 7 days post fertilization (dpf) were mounted in agarose gel with a layer of low-melting agarose on top. Time-lapse videos were captured at 5 fps to obtain the quantitative measures of motility using a custom-Matlab algorithm – Particle Image Velocimetry (PIV) Lab. The measures that can be extracted included frequency, amplitude, velocity magnitude, and vector directions of designated regions of interest (ROI) on the intestinal muscles. From a preliminary study, we constructed wave propagation maps to indicate the velocity differences of the intestinal contractions between initial feeding samples at 5 dpf, 6 dpf and 7 dpf respectively using spatiotemporal maps (STMs). Our initial data from 6 animals of each samples showed contractile waves in both dorsal-ventral and anterior-posterior maps, with the mid-intestine, posterior region and fed siblings showing more prominent velocity spikes. We aim to fully characterise GI motility in zebrafish larvae before investigating GI motility disorders in several disease models. We also aim to develop a novel platform for screening novel chemical entities acting on the GI tract.

CBET8

Enhancer dysregulation of myeloid-derived suppressor cells in hepatocellular carcinoma

Wing Yan LAW, Man LIU, Jingying ZHOU, Ka Wing CHEUNG

Supervisor: Alfred Sze Lok CHENG

Numerous large-scale prospective population-based studies have consolidated the significant correlation of liver fibrosis and hepatocellular carcinoma (HCC). Our most recent findings demonstrated that myeloid-derived suppressor cells (MDSCs), especially the monocytic subtype (M-MDSCs), was dramatically expanded in fibrous liver microenvironment in mouse models and HCC patients (Liu et al., *Gut* 2020). As the predominant immunosuppressive cells in HCC, M-MDSCs potently suppressed anti-tumor immune responses and promoted hepatic tumorigenicity. Mechanistically, we uncovered that enhancers, key for cell identity determination, played a pivotal role in triggering monocyte to M-MDSC development and immunosuppressive function. We further found that enhancer inhibition by bromodomain and extraterminal domain (BET) inhibitors that disrupt bromodomain protein 4 (BRD4)/histone lysine 27 acetylation (H3K27ac) interaction could abrogate M-MDSC generation and activation. Targeting MDSC by BET inhibitor *in vivo* led to tumor reduction and prolonged host survival in fibrosis-associated HCC mouse model. Given the key role of enhancer remodelling in M-MDSC, we aim to further elucidate the specific enhancer targets that promote MDSC identity and function in the immunosuppressive liver microenvironment.

Using single cell-RNA sequencing (scRNA-seq) from HCC patient samples and bulk RNA sequencing from *in vitro*-derived M-MDSCs, 13 signature genes that exhibit aberrant overexpression in M-MDSC were identified, including a soluble ligand of epidermal growth factor receptor (EGFR). Transcriptomic profiling in conjunction with FANTOM5 (Functional Annotation of the Mammalian Genome) database and JEME (Joint Effects of Multiple Enhancers) algorithm revealed specific enhancer RNA (eRNA) expressions that control the up-regulation of the EGFR ligand in M-MDSC. The enhancer regulation was further validated by H3K27ac occupancy through chromatin immunoprecipitation (ChIP)-qPCR. Importantly, we found that this EGFR ligand was highly expressed in HCC patient-derived M-MDSCs as shown by intracellular flow cytometry, scRNA-seq and co-immunofluorescence analysis, highlighting the clinical relevance of our findings. The knockdown of eRNA of this ligand significantly downregulate its mRNA level. In summary, our findings identified a novel enhancer-regulated target that might uncover new immunosuppressive mechanism and MDSC-directed strategy for cancer immunotherapy.

CBET9

Integrated analysis of differential expressed genes and somatic variants in transcriptomic data of prostate cancer patients reveals potential diagnostic and prognostic markers

LIANG Yonghao, TSUI Kwok-Wing Stephen

Supervisor: TSUI Kwok-Wing Stephen

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR;

Background: Despite recent advances in the diagnosis and treatment of prostate cancer, the overall prognosis and survival rates for prostate cancer remains low. With the recent advances in next-generation sequencing technologies, researchers have made use of whole genome sequencing to identify genetic variants or RNA sequencing to find differentially expressed genes (DEGs). Given the situation that researchers seldom combine variants and DEG information from transcriptomic data, we hypothesized that systematically integrating the analysis of DEGs and somatic mutations may reveal novel and promising markers for diagnosis and prognosis in prostate cancer.
Materials and Methods: To tackle this question, we analyzed 60 pairs of transcriptomic data from prostate cancer patients by a bioinformatics pipeline integrating HISAT 2/StringTie/DESeq 2 and GATK/SIFT4G. The data called by the pipeline were further validated in public datasets by GEPIA and cBioPortal.
Results: Totally, 1417 intolerant genes were called by the combination of GATK and SIFT after deducting 891 overlap intolerant genes between prostate cancer and adjacent normal groups. At the same time, 73 DEGs were elucidated by HISAT 2/StringTie/DESeq 2 pipeline. By integrating results of 73 DEGs and 1417 somatic mutations, novel candidate markers *NOC2L* and *GPRASP1* were identified. Using the public datasets from TCGA and GTEx with 492 prostate cancer samples and 152 normal prostate tissue samples for validation by GEPIA, the expression level of *NOC2L* was found to be significantly higher in prostate cancer compared with the paired normal samples while the expression level of *GPRASP1* is significantly lower in prostate cancer compared with the paired normal samples. For either NOC2L or GPRASP1, the patient group having this gene mutation has more copy number variation and snp/indel mutation compared with unaltered group, which is confirmed in a 5000-patient cohort by cBioPortal. *NOC2L*, which can act as a transcription corepressor of p53/TP53- and TP63-mediated transactivation of the p21/CDKN1A promoter, may be involved in the regulation of p53/TP53-dependent apoptosis. *GPRASP1*, which is a tumor suppressor, has been reported to have differential methylation in prostate cancer. Moreover, *NOC2L* was identified as a prognostic marker of prostate cancer patients. A higher expression of *NOC2L* was significantly correlated with the worse diseasefree survival with a hazard ratio of 1.8, which was confirmed by a TCGA dataset with 344 prostate cancer patients. Patients having mutation of *GPRASP1* tend to have a significant better survival time compared with the group without this mutation, which is confirmed in a 664-patient cohort by cBioPortal.
Conclusion: Our study identified novel candidate markers *NOC2L* and *GPRASP1* for prostate cancer by integrated analysis of DEGs and somatic variants in transcriptomic data. These markers were further validated in public

datasets by GEPIA and cBioPortal. Our findings can shed light on the better diagnosis and prognosis in prostate cancer.

CBET10

Investigation of PA-induced DNA damage and repair response in HepaRG cells and rat livers

Yun LONG, Yisheng HE

Supervisor: Ge LIN

Pyrrolizidine alkaloids (PAs) are group of the most common phytotoxins produced by more than 6,000 plant species worldwide. PAs have been reported to be mutagenic and tumorigenic in rodents. However, the biochemical mechanism underlying PA-induced DNA damage remains largely unknown. In this study, we demonstrated that representative toxic PAs, including retrorsine (RTS), monocrotaline (MCT) and clivorine (CLI), induced expression of γH2AX, a marker of DNA damage, in HepaRG cells (a human liver cell line) in both time and dose dependent manner. Among three PAs tested, RTS showed the highest genotoxicity. Using RTS, we then investigated the DNA repair responses to PA-induced DNA damage. We found that RTS exposure caused expressions of Rad51 and BRCA1 in HepaRG cells and rat livers, indicating that homologous recombination (HR) repair pathway was involved in repairing RTS-induced DNA damage. Besides, RTS-exposed HepaRG cells and rat livers demonstrated significant expression of KU70 and DNA-PKcs which are markers of non-homologous end joining (NHEJ) repair pathway. Taken together, we have demonstrated that different PAs commonly caused DNA damage in HepaRG cells, and HR and NHEJ pathways were involved in repairing RTS-induced DNA damage in HepaRG cells and rat livers. [Supported by Research Grants Council of HKSAR (GRF Grant No. 14107719) and The Chinese University of Hong Kong (Project Impact Enhancement Fund (PIEF) (3133029)]

CBET11

Investigation of pyrrolizidine alkaloid-induced liver injury in zebrafish

Yueyang PAN, Jiang MA, Hui ZHAO, Ge LIN

Supervisor: Ge LIN

Pyrrolizidine alkaloids (PAs) are phytotoxins identified in over 6000 plant species in the world. Humans are readily exposed to toxic PAs through the consumption of PA-producing plants used as herbal medicines and teas or by the unwitting ingestion of PA-contaminated foodstuffs. Various authorities, such as the Australia New Zealand Food Authority (ANZFA) and the European Food Safety Authority (EFSA), have established different exposure thresholds based either on human cases of acute hepatic sinusoidal obstruction syndrome or carcinogenicity studies with lasiocarpine/riddelliine in rodents to restrict the PA exposure. Nevertheless, all those regulations assume an equal toxic potency of structurally diverse PAs. However, different PAs have been demonstrated to exhibit significantly different toxic potencies. Therefore, the understanding of toxic potencies of structurally diverse PAs would facilitate the further risk assessment of PA exposure and the establishment of more appropriate regulations. In the present study, a zebrafish model was used for evaluating the toxic potency of different PAs, because it is an *in vivo* model that can describe the absorption, distribution, metabolism, and excretion processes and it possesses a wide range of cytochrome P450 enzymes that are responsible for metabolic activation of PAs to exert their toxicity. Biochemical analysis and histological examination were performed in zebrafish orally administered with 0.3 mmol/kg of nine structurally diverse PAs or the same volume of vehicle control for 6 hours. The results revealed that compared to the control group, zebrafish in PA-treated groups exhibited obvious liver injuries, evidenced by obvious histological changes in liver slices stained by H&E and oil red O, significantly elevated ALT activity and the formation of pyrrole protein adducts. Moreover, structurally different PAs indeed caused distinct hepatotoxicity in zebrafish, with the order of lasiocarpine ~ retrorsine > monocrotaline > riddelliine > clivorine > heliotrine > retrorsine *N*-oxide ~ riddelliine *N*-oxide >> platyphylline. These findings demonstrated that zebrafish is a promising model for screening and ranking the toxicity of PAs with different structures, which can facilitate future establishment of more appropriate regulations of PA intake.

[Supported by The Chinese University of Hong Kong (Project Impact Enhancement Fund (PIEF) (3133029)]

CBET12

Characterization of novel allergens in storage mites

S.K. Shin[1], Q. Xiong[1], C.C. Lau[1], M. Nat[2], S.K.W. Tsui[1]

Supervisor: S.K.W. Tsui[1]

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Blomia tropicalis (*B. tropicalis*) is a species of storage mites which are frequently found in indoor space and therefore highly related to human health. Especially, they are commonly found in the homes of tropical and subtropical regions of the world, and usually found in stored food. They are one of the important sources of allergens in domestic environment. There are 14 groups of *B. tropicalis* allergens which are officially acknowledged up to date but it is predicted that there are more allergens to be identified. The study of allergens is crucial because the human allergic diseases such as allergic rhinitis, asthma and atopic dermatitis could be attributed to the exposure to the allergens from body and faeces *B. tropicalis*. In fact, it has been reported that there was an increased sensitization rate to *B. tropicalis* in the past few years. Hence, there is an increased demand for a complete profile of allergens of *B. tropicalis* which is fundamental to the comprehensive diagnosis and therapy of these allergic diseases.

In this study, the identification of novel allergens in *B. tropicalis* has been carried out utilizing the high-quality genome assembly of *B. tropicalis*. 55 newly identified allergens belonging to 23 novel groups were found which require characterization. Among these novel allergens, a few have been expressed and purified. They need to be further investigated for their allergenicity in order to be fully recognized as *B. tropicalis* allergens. Besides, the study on epitope mapping of allergens can help in the discovery and development of new therapeutics, vaccines, and diagnostics for allergies.

CBET13

Resistance to immune checkpoint targeting in hepatocellular carcinoma: Role of tumor interferon signaling

TU Yalin, Alfred Sze-Lok Cheng

Supervisor: Alfred Sze-Lok Cheng

Immune checkpoint blockade (ICB) therapies by antibodies have revolutionized the treatment paradigm for a variety of cancers. Although subsets of people exhibit durable responses, resistance and relapse are common and influenced by factors inherent to immune cells and cancer cells. While the level of T cell infiltration in the tumor microenvironment (TME) can impact ICB outcome, key cancer cell-intrinsic features include their neoantigen repertoire, major histocompatibility complex class one (MHC-I)-mediated antigen presentation, and the expression of inhibitory receptor ligands such as programmed death-ligand 1 (PD-L1) also play important roles. However, it remains challenging to overcome ICB resistance due to the lack of knowledge on cancer cell evolution with respect to T cell interactions upon immunotherapy.

Previously, we have recapitulated the clinical outcome of ICB resistance via repeated cycles of *in vivo* selection in orthotopic-grafted murine models of HCC. As a result, anti-PD-L1 therapy failed to reduce tumor growth. Strikingly, our single-cell transcriptome analysis revealed that >90% of the differentially-expressed genes in the ICB-resistant HCC cells were significantly reduced, whose top functional enrichment was the response to interferon-gamma (IFNγ), a cytokine with critical roles in MHC-I-mediated antigen presentation, T cell-mediated cytotoxicity and PD-L1 expression. Here, we hypothesized and have partially verified that deficient hepatoma-intrinsic IFNγ signaling confers ICB resistance through insensitivity to the cytotoxic T lymphocytes (CTLs) and immunosuppressive TME reprogramming. Notably, we observed global histone deacetylation in ICB-resistant HCC cells, which led to reduced IFNγ-stimulated gene expressions and cytotoxicity. We will further delineate the epigenetic mechanisms underlying cancer cell evolution to identify potential ICB-sensitizing therapeutic targets.

CBET14

Investigating the HDAC8-mediated 3D chromatin conformation in T cells: role in cancer immunotherapy

Jing Wang, Jingying Zhou, Yu Feng, Alfred Sze-Lok Cheng

Supervisor: Alfred Sze-Lok Cheng

The limited efficacy of immune checkpoint blockade (ICB) in hepatocellular carcinoma (HCC) is associated with the insufficient infiltration of $CD8^+$ T cells into tumor microenvironment. Combinational therapy such as epigenetic inhibitors has brought light into overcoming the obstacle. Aberrant expression of histone deacetylase 8 (HDAC8) in several cancers has been well studied. Our previous study also shows HDAC8 is an oncogene in HCC. In our more recent study, pharmacologic inhibition of HDAC8 has been uncovered to promote tumor-infiltration $CD8^+$ T cells and exhibits large tumor eradication in combination with anti-PD-L1. The co-blockade of HDAC8/PD-L1 can also induce durable protection against re-challenged tumor through increased memory T cells.

The non-histone substrate of HDAC8, Structural Maintenance Of Chromosomes 3 (SMC3), is the component of ring-shaped cohesin, which is involved in topologically entrapping enhancers and promoters to modulate gene expression. While selective inhibition of HDAC8 on tumor cells has been well illustrated, but whether it has a role in T cells is unknown. Here we demonstrate that specific inhibitor of HDAC8 PCI-34051 significantly increases the acetylation of SMC3 in mouse $CD4^+$ T cells, which is associated with upregulation of $CD4^+$ T signature genes such as key memory inducing cytokines IL-15 and IL-2. This is accordant with our previous finding of memory $CD8^+$ T cells induction in mouse HCC model upon anti-HDAC8/PD-L1 combinational immunotherapy. We anticipate that the analysis of genome-wide SMC3-mediated promoter-enhancer interactions upon selective HDAC8 inhibition in $CD4^+$ T cells may help understand $CD4^+$ T cell 3D epigenome and better develop anti-tumor immunity for cancer immunotherapy.

CBET15

Transcriptome immune signatures of ALK-altered HNSCC

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase encoded by the gene *ALK*, which belongs to a subfamily of the insulin receptor superfamily. Recently, *ALK* aberrations, including gene fusions and point mutations, are known to be involved in the oncogenesis of non-small cell lung cancer and glioblastoma. Head and neck cancer squamous cell carcinoma (HNSCC) is an aggressive cancer with genomic heterogeneity as revealed by recent whole-exome studies. Though *ALK* aberrations are found in a notable subset of HNSCC, their biological importance is largely unknown.

Comprehensive pan-cancer analysis of 33 cancer types (TCGA, Pan-cancer, Provisional, N=11617, www.cbioportal.org) showed that *ALK* aberrations (mutations, amplification and gene copy gain) are commonly noted in 27 cancers, with HNSCC ranking 9th having ~22% cases of primary tumors harboring these *ALK* genomic aberrations. Within the US TCGA HNSCC dataset (Provisional; N=510), 3.5% of HNSCC tumors harbor somatic *ALK* mutations, 0.4% cases with *ALK* amplification, and 17.4% cases with *ALK* copy number gain. Further, RNA-seq analysis revealed tumor specific *ALK* mRNA upregulation in 43 pairs of normal-tumor tissues (~2-fold, P=0.001), and 457 HNSCC tumors (~1.5-fold, P=0.039). Further, in our in-house NGS Asian HNSCC patient cohort (N=114), we noted a 4.4% rate of *ALK* somatic point mutations (5/114 cases), as well as an 18.4% rate of germline variants (21/114 cases) with unknown clinical significance.

Interestingly, *ALK* aberrations (mutations, amplification, gene copy gain) are noted to be significantly associated with *TP53* mutations (P<0.0001), HPV-negativity (P=0.0094) and the male gender (P=0.0041; Fisher's Exact test), but not associated with HNSCC patient survival (P=0.734). Further, patients with *ALK* mutation and amplification are largely advanced cases (93.33%, Stage III and Stage IV). Gene Set Enrichment Analysis (GSEA) of mRNA expression shows that *ALK*-altered HNSCC tumors (vs unaltered tumors) appear to have an overall downregulation of immune response gene set [NES (normalized enrichment score)=-6.773, P=0.0026] and inflammatory response gene set (NES=-5.54, P=0.0022), while nervous system development and cell

development signaling gene sets are significantly upregulated (P=0.0015, 0.0016, respectively). Moreover, tumor immune estimation resources (TIMER) analysis reveals significant decreases of CD8+ T cell, neutrophils and dendritic cell infiltration levels in ALK-altered HNSCC tumors vs unaltered tumors (P=0.025, 0.00009, 0.0333 respectively). In conclusion, the immunosuppressive transcriptome signature of *ALK*-altered HNSCC may have biological implications for their tumorigenesis, which warrant future investigations.

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CBET16

Effect of diet intake on the gut microbiome composition of allergic patients and dynamic structural process of N-cycle functional modules.

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Allergy, also known as allergic diseases, including food allergies, atopic dermatitis, allergic asthma, anaphylaxis and hay fever, have significantly elevated in prevalence over of last few decades. Recent research points to a central role of the microbiome, which is highly influenced by multiple environmental and dietary factors. It is well established that the microbiome can modulate the immune response, from cellular development to organ and tissue formation exerting its effects through multiple interactions with both the innate and acquired branches of the immune system. It has been described at some extent changes in environment and nutrition produce dysbiosis in the gut but also in the skin, and lung microbiome, inducing qualitative and quantitative changes in composition and metabolic activity. Here we combine network structure analysis and microbial function analysis to reveal the modifications in the composition and structure of gut microbiome in no-allergy people, allergies, repeated allergies and allergies cured from a new perspective. 192 fecal samples were subjected to 16S rRNA sequencing. The raw reads were processed by quality filtering, diversity analysis, taxonomical as well as functional profiling and network analysis using Qiime2 and related R packages. Dietary intake information such as cereal intake ratio, vegetable intake ratio, fruits in take ratio, milk and dairy intake ratio, and meat, fish, eggs and beans intake ratio were also collected at the same time. Eventually, significant difference in alpha diversity and beta diversity were detected between allergy and no allergy group. 13 genera of bacteria, and 3 species were enriched in the gut microbiome of the non-allergic group. 1 family of bacteria, 7 genera and 2 species were enriched in the gut bacteria of the allergic group. Besides, there was dramatic positive correlation between daily vegetable intake ratio and species diversity, and significant negative relationship was detected between daily milk and dairy products intake ratio and gut microbiome local diversity. Furthermore, according to network analysis and functional profiling prediction, we can see the dynamic process of the structure and function of gut microbiome from normal people to allergies, repeated allergies and allergies cured. In no-allergy group, modules with N-cycle account for less and are not in the center of the network; In allergy group, more OTUs in modules with N-cycle have become important hub nodes of the microecological network; In repeated allergies, many OTUs of modules with N-cycle have become the hub nodes of the network to maintain the stability of the network

structure; While in allergy cured group, modules with N-cycle is no longer in an important network hub position.

CBET17

The causal inference among hypertension, antihypertensive drugs and osteoporosis Using Mendelian randomization study

XIANG Yong, SO Hon-Cheong

Supervisor: Hon-cheong So

Background: The causal relationship between hypertension (HT) and osteoporosis (OP) remains unclear. The effects of the different antihypertensive drugs on bone health are inconclusive. We aim to determine whether hypertension and antihypertensive drugs casually affect bone health by combing the epidemiological analysis and mendelian randomization (MR) study.
Methods: The association between blood pressure (BP) and bone mineral density (BMD) and the association among antihypertensive drugs and BMD, the risk for osteoporosis/fracture were examined both in two large cohorts of the National Health and Nutrition Examination Survey (NHANES) and UK Biobank (UKBB). The causality among HT, antihypertensive drugs, and BMD were inferred by two-sample MR approach utilizing summarized genome-wide association study (GWAS) data. Genetic proxies for each antihypertensive medication were used to infer its causal estimate on BMD.
Results: In the epidemiological observational studies, the null association was found between BP and BMD. The use of thiazide diuretics was significantly associated with 0.08 SD (standard deviation), 0.08 SD, and 0.14 SD BMD increases at femur neck, total femur, and total spine respectively (P = 5.43E-04; 2.45E-04; 3.70E-09). While the treatment with loop diuretics was associated with 0.19 SD BMD decrease at the hip. The combination treatment of beta-blockers and calcium channels blockers (CCBs) was also observed a detrimental effect on BMD at the hip. The MR methods indicated that the null causal association exists between BP and BMD. The results from drug target analysis revealed that the use of thiazide diuretics may be genetically predicted 0.04 SD increase in BMD (P = 0.025). While the treatment of beta-blockers or CCBs may have a detrimental causal association with BMD.
Conclusions: Null causal association exists between BP and BMD. Thiazide diuretics may causal protective bone health, while loop diuretics, beta-blockers, and CCBs may be potentially detrimental to bone metabolism.

CBET18

Integrating Clinical Data and Imputed Transcriptome from GWAS to Uncover Complex Disease Subtypes: Applications in Psychiatry and Cardiology

Liangying Yin, Hon-cheong So

Supervisor: Hon-cheong So

Classifying subjects into clinically and biologically homogeneous subgroups will facilitate the understanding of disease pathophysiology and development of targeted prevention and intervention strategies. Traditionally, disease subtyping is based on clinical characteristics alone, but subtypes identified by such an approach may not conform exactly to the underlying biological mechanisms. Very few studies have integrated genomic profiles (e.g., those from GWASs) with clinical symptoms for disease subtyping. Here we proposed an analytic framework capable of finding complex diseases subgroups by leveraging both GWAS-predicted gene expression levels and clinical data by a multi-view bicluster analysis. This approach connects SNPs to genes via their effects on expression, so the analysis is more biologically relevant and interpretable than a pure SNP-based analysis. Transcriptome of different tissues can also be readily modeled. We also proposed various evaluation metrics for assessing clustering performance. Our framework was able to subtype schizophrenia subjects into diverse subgroups with different prognosis and treatment response. We also applied the framework to the Northern Finland Birth Cohort (NFBC) 1966 dataset and identified high and low cardiometabolic risk subgroups in a gender-stratified analysis. The prediction strength by cross-validation was generally greater than 80%, suggesting good stability of the clustering model. Our results suggest a more data-driven and biologically informed approach to defining metabolic syndrome and subtyping psychiatric disorders. Moreover, we found that the genes "blindly" selected by the algorithm are significantly enriched for known susceptibility genes discovered in GWASs of schizophrenia or cardiovascular diseases. The proposed framework opens up an approach to subject stratification.

Keywords: disease subtyping, clustering; genome-wide association study; gene expression

DEVELOPMENTAL AND REGENERATIVE BIOLOGY

Developmental and Regenerative Biology

Title	Author	Abstract Number
The role of hyaluronic acid in the assembly of a mesenchymal stem cell-derived extracellular matrix showing anti-inflammatory potential	Assunção Marisa	DRB1
Carbon monoxide attenuates oxidative stress-induced senescence in human airway epithelial cells	CAI Mengyun	DRB2
Differentiation of Epidermal Stem Cells from iPSC	Chan See Wing	DRB3
Post-transcriptional modifications in oocyte during maturation and maternal aging	CHAN Ting Hei	DRB4
Study the roles of NGF in human cartilage with 3D pellet culture system	CHAN Yau Tsz	DRB5
Machine learning algorithms applied to Raman Spectroscopy for investigation of myogenic differentiation	CHENG Chien Ting	DRB6
Study of Dual Functions of β-catenin during Definitive Endoderm Differentiation of Human Embryonic Stem Cells	DAI Liujiang	DRB7
Comparing 2D Versus 3D culture of Hepatoyctes in Different ECM environment for Liver Tissue Engineering	DENG Shuai	DRB8
The function of a primate-specific microRNA-1202 in dopaminergic neurons	HAO Suyu	DRB9
Bioengineering 3D Printed Auricular Scaffold for Microtia Treatment	HUANG Na	DRB10
Non-collagen proteins are essential for the pro-tenogenic bioactivity of tendon extracellular matrix on human adipose stem cells	HUANG Shuting	DRB11
Thyroid hormone regulates heart regeneration in zebrafish	JIANG Chunmei	DRB12
The physiological functions of β-defensin 19 in the testis	JIN Jing	DRB13
INDUCED REJUVENATION OF MESENCHYMAL STEM CELLS DERIVED FROM AGED HUMAN ADIPOSE TISSUE USING SMALL MOLECULE SC1231	Laane Grete	DRB14
Improving oocyte quality through mitochondrial transfer from mesenchymal stem cells via tunneling nanotubes	LEE Wing Tung	DRB15
Induction and transplantation of neural crest cells from mouse induced pluripotent stem cells	LI Dandan	DRB16
Growth factor-based, multi-functional regeneration for muscle and tendon in chronic massive rotator cuff tears	LI Ke	DRB17

Mechanistic study of transcription factor SOX18 during heart development	LIANG Jianxin	DRB18
The Potential Role of RSPO3 in Oligodendrogenesis	LIN Jiacheng	DRB19
Identification of essential factors during *Xenopus* heart regeneration	LIN Yi-jyun	DRB20
Developing a molecular age model for oocyte assessment	NG Kin Wing	DRB21
The role of KDM3A in vascular function and regeneration: implication of aging	Qin Weifeng	DRB22
Single cell ATAC-Seq reveals epigenetic heterogeneity associated with an EMT-like process in male germline stem cells	SUEN Hoi Ching	DRB23
A Physiological Dynamic Mechanical Loading Platform for Meniscus Tissue Engineering	SUN Jing	DRB24
NAD+ Supplement Rescues Adipogenesis and Metabolism in Werner Syndrome	TIAN Yuyao	DRB25
In vitro 3D liver model utilizing iPSC induced hepatocytes for drug testing	Tsang Hoi Ying	DRB26
Development of Gene Therapy for β-Thalassaemias through Therapeutic Genome Editing in Human Blood Stem Cells	URIP Brian Anugerah	DRB27
Development of an in vitro hematopoietic stem cell niche on-a-chip	WAN Ho Ying	DRB28
Comparison of migration and differentiation of enteric neural crest cells and hair follicle-derived neural crest stem cell both in the hindgut explant cultured ex vivo and in the distal colon of adult mouse *in vivo*	ZHANG Yan	DRB29
CRISPR/Cas9 mediated knock-in for the treatment of inherited hemophilia	ZHANG Zhenjie	DRB30
Novel Intrinsic and Extrinsic Regulation of Spermatogonial Stem Cells Maintenance	ZHENG Tingting	DRB31
Functional characterization of a hydrogel-based bioink for fabrication of engineered cartilage like tissue	ZHOU Zheyuan	DRB32
Modulation of macrophages by bioactive glass/sodium alginate hydrogel is crucial in skin regeneration enhancement	ZHU Yanlun	DRB33
Microfluidic chips as a 3-dimensional experimental tool for studies of single cell migration	ZHU Yu	DRB 34

DRB1

The role of hyaluronic acid in the assembly of a mesenchymal stem cell-derived extracellular matrix showing anti-inflammatory potential

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Cell-derived extracellular matrix (CD-ECM) can be used as a cell-free, natural biomaterial capable of modulating complex cellular and physiological processes (i.e. tissue healing). By adjusting the culture conditions, CD-ECM can attain specific bioactivity. This work aimed to produce a CD-ECM with anti-inflammatory properties applicable to the treatment of chronically inflamed tissues, such as those in chronic wounds. Mesenchymal stem cells (MSCs) were the chosen ECM producers, due to their known anti-inflammatory paracrine activity. High molecular weight hyaluronic acid (HA), a highly pro-regenerative and anti-inflammatory macromolecule, was used in MSCs' cultures. We hypothesized that the anti-inflammatory features of MSCs secretome and HA would be incorporated into the assembled CD-ECM, translating into a CD-ECM with enhanced anti-inflammatory properties.

Bone marrow MSCs were cultured in the presence of HA (0, 5, 50, 500 and 1000 μg/ml) between 2 and 6 days. The effect of HA on fibronectin (FN) deposition, one of the main components of MSCs' ECM and one of the first to be deposited, was assessed by immunocytochemistry and revealed that increasing HA concentration correlated with higher FN deposition, especially at day 2. A thorough investigation by confocal microscopy placed HA in the FN fibrillogenesis machinery. On the other hand, degradation of HA disturbed FN fibril assembly, proving its importance in early ECM deposition.

To verify if the HA-improved ECM (HA-CD-ECM) was anti-inflammatory, the CD-ECM was isolated and used as a substrate for macrophages pulsed with pro-inflammatory factors (lipopolysaccharide and interferon-γ). HA-CD-ECM prevented the release of a major pro-inflammatory molecule (tumor necrosis factor-α; 36+/-26 pg/ml), in comparison to macrophages seeded on bare plastic (343+/-43 pg/ml), on control CD-ECM (173+/-54 pg/ml) and macrophages not exposed to pro-inflammatory stimulus (65+/-35 pg/ml).

In conclusion, HA is a multifactorial tool for the formulation of 1) more robust CD-ECM by regulating FN fibril formation and 2) a CD-ECM with potentiated anti-inflammatory properties.

DRB2

Carbon monoxide attenuates oxidative stress-induced senescence in human airway epithelial cells

Meng-yun CAI, Chung-Yin YIP, Xiaoqiang Yao, Wing-hung KO

Supervisor: Wing-hung KO and Xiaoqiang Yao

Cellular senescence is characterized by irreversible arrest of cell cycle progression, and it occurs either after successive rounds of cellular replication (replicative senescence) or when triggered by exogenous and endogenous stress and damage (premature senescence). In response to potential threats, airway epithelia can recognize "danger signals" and respond quickly and appropriately to repair the defects resulting from injury. However, continued or overloaded stimulus from inhaled toxins may cause oxidative stress and DNA damage and further lead to stress-induced senescence in epithelial cells, which can contribute to the pathogenesis of several airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Over the last decade, carbon monoxide (CO) has been shown to confer cytoprotective effects, such as anti-oxidation, anti-inflammation, and anti-apoptosis. This study therefore investigated the effects of CO on oxidative stress-induced senescence in human airway epithelial cells and the underlying cellular mechanisms.

In the study, two human airway epithelial cell lines, 16HBE14o- and BEAS-2B, were used to establish a hydrogen peroxide (H_2O_2)-induced senescence model. After treating the epithelial cells with carbon monoxide-releasing molecule-2 (CORM-2) or a CO-containing solution, the intracellular CO concentration was monitored by a Nile red CO fluorescent probe. We observed a concentration- and time-dependent increase of fluorescence signals in the cells after CO treatment. Quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting were used to evaluate the expression of $p21^{Cip1}$, a potent marker of cellular senescence, at the mRNA and protein level, respectively. Exposure of epithelial cells to H_2O_2 increased the expression of $p21^{Cip1}$ in a concentration-dependent manner, which was significantly attenuated in the presence of CO. A cell-permeable fluorescent indicator, carboxy-H_2DCFDA, was used to measure the intracellular concentration of total reactive oxygen species (ROS). It was shown that the H_2O_2-mediated increase in intracellular total ROS production was reduced by CO treatment. These data suggest that CO could attenuate epithelial senescence by, at least in part, inhibiting the generation of intracellular ROS. Further studies are required to explore the underlying mechanisms and the signaling pathway by which CO affects epithelial senescence.

DRB3

Differentiation of Epidermal Stem Cells from iPSC

See Wing Chan, Hoi Hung Cheung, Wai Yee Chan

Supervisor: Wai Yee Chan

The objective of the project is to establish an inhouse protocol for generating human epidermal stem cells (EpSCs) from iPSC. Skin composes of several components, including interfollicular epidermal cells, sweat glands, hair follicles and sebaceous glands to function as physical barrier in defense mechanism and body temperature regulation. EpSCs are found at the basal layer in epidermis and bulge region near hair follicle. These EpSCs are responsible for maintenance and healing of skin.

Although skin process an extent of regenerative potential to provide defense as physical barrier under normal circumstances, loss of EpSCs due to aging, third-degree burn and genetic disorder would lead to partial skin degeneration and make patients susceptible to microbial infection.

There are three approaches to study skin substitutes, including direct differentiation from iPSC to EpSCs, co-culture of iPSC-keratinocytes and iPSC-fibroblasts, and skin organoids from embryoid bodies. While direct differentiation approach provides direct study of the EpSCs, co-culture and organoid approaches allow study of interaction between cell types.

Last year's experiment focus on the direct differentiation approach. Depending on Yang's finding in 2014, we explored different factors on the differentiation of EpSCs from iPSC. Feeder cells density, embryoid body density, presence of feeder cells, coating matrix were tested in different trials. Detachment of cells clumps and failure in passage attachment were encountered. Immunostaining and qPCR analysis were performed on Day 18 differentiated EpSCs showed only small population met the required CD200+/ITGA6+ EpSC population. This suggests further testing are required to establish the protocol of differentiation.

DRB4

Post-transcriptional modifications in oocyte during maturation and maternal aging

TTH Chan, MY CHEUNG, JKW NG, WT LEE, HC SUEN, HT CHU, J LIAO, ACS LUK, YL CHAN, TL LEE

Supervisor: TL LEE

RNA modification plays an important role in shaping the complexity of proteome in different tissue and organs. Here we show the transcriptional dynamics during oocyte maturation and maternal aging. Differentially spliced genes led to an increase in functional proteins during oocyte maturation, while maternal aging reverses the rise. On the other hand, alternative polyadenylation analysis demonstrated genes related to chromatin and histone modifications are lengthened, while genes related to energy metabolism are shortened in aging. We found that m6A motif is enriched upstream of the alternative polyadenylated site. Here, we hypothesize that m6A regulates the alternative polyadenylation and maternal mRNA degradation, and contributes to decrease in oocyte quality during aging.

DRB5

Study the roles of NGF in human cartilage with 3D pellet culture system

CHAN Yau Tsz, SUN Jing, HO Ki Wai Kevin, TUAN Sung-chi Rocky, JIANG Yangzi

Supervisor: TUAN Sung-chi Rocky, JIANG Yangzi

Osteoarthritis is a degenerative joint disease characterized by loss of articular cartilage. Patients will suffer from pain, movement limitation, and even disability. Increased nerve growth factor (NGF) expressions in synovial fluid and cartilage are observed in osteoarthritis patients, therefore we hypothesized that NGF plays a role in osteoarthritis. This study aimed to investigate the roles of NGF in human cartilage using a 3D pellet culture system. In this study, chondrocytes were isolated from human knee articular cartilage from total knee joint arthroplasty (CREC NTEC Ref. 2019.078) and two batches of pooled chondrocytes were used. Chondrocyte 3D pellets were cultured in different culture media with or without the treatment of NGF or Nsp (NGF small peptide). The pellet size, cell morphology, and extracellular matrix content of the harvested pellets were analysed. Results showed that chondrogenic media could significantly increase the pellet size. We also found that treatment of NGF or Nsp did not consistently significantly affect the pellet size or neocartilage properties in different batches, thus further study with more biological donors is required.

DRB6

Machine learning algorithms applied to Raman Spectroscopy for investigation of myogenic differentiation

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Raman spectroscopy is a non-invasive, label-free, rapid tool and has been used as a fingerprint technique for the detection and identification of chemical compounds as well as biomedical samples. Myogenic differentiation can be identified by the distinct morphology of elongated, cylindrical, multinucleated myofibrils, or assessed by numerous biological assays including reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence staining. However, conventional methods to evaluate the myogenic differentiation may be insufficient, time-consuming and cell-destructive. In this study, we developed an approach for using Raman spectroscopy to perform real-time monitoring of myogenic differentiation. We collected Raman spectra of C2C12 cell line and human muscle derived cells (hSkMDC) undergoing myogenic differentiation and assessed Raman signals changes during myogenic differentiation for C2C12 myoblasts and hSkMDC. Such comparisons confirmed the versatility of our approach for multiple myogenic cells that range from immortalized mouse cell line to human primary cells. Furthermore, we are developing a machine learning algorithm to rapidly process Raman spectra and provide robust, label-free detection of myogenesis. Myogenic differentiation of C2C12 and hSkMDC were confirmed by RT-PCR and IF staining. The results demonstrated that there was a significant distinction in Raman spectra in different stages of myogenic differentiation. Increase in the intensities of peak at 748 cm^{-1}, 1127 cm^{-1} ,1310 cm^{-1}, and 1558 cm^{-1} characteristic of cytochrome C, and 1245-1345 cm^{-1} refer to myosin during formation of myotubes can be observed.

In summary, we have demonstrated the ability of Raman spectroscopy to detect molecular changes related to myogenic differentiation which could be used as Raman biomarker and established the fingerprint for myogenesis. Application of Raman spectroscopy combined with machine learning facilitate label-free detection of myogenesis, which could broadly applied as a versatile research tool to investigate myogenesis, muscle regeneration, drug treatment, clinical diagnosis, and cell therapy.

DRB7

Study of Dual Functions of β-catenin during Definitive Endoderm Differentiation of Human Embryonic Stem Cells

Liujiang DAI, Xun MA, Jiangchuan LI, Chunlai TAN, Xiangjun HE, Chenzi ZHANG, Zhenjie ZHANG, Brian ANUGERAH, Jingyi WANG, Bo FENG

Supervisor: Bo FENG

β-catenin is a key effector in the canonical Wnt pathway, and it also regulates cell adhesion at the plasma membrane. It remains controversial which function of this protein is required during differentiation of hESCs. By overexpressing different β-catenin mutants in β-catenin KO hESCs, we showed that Definitive endoderm (DE) differentiation potentials of these mutants did not correlate with their transcriptional activities. A mutant β-catenin (ΔNC-β-catenin) without N terminal or C terminal could still rescue DE differentiation. Dysfunction of cell adhesion of β-catenin by knock out (KO) of E-cadherin or knock out (KD) of α-catenin did not impair DE differentiation. ΔNC-β-catenin could still enter nucleus during DE differentiation.

DRB8

Comparing 2D Versus 3D culture of Hepatoyctes in Different ECM environment for Liver Tissue Engineering

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Cells are surrounded by extracellular matrix (ECM) which influences cell proliferation, differentiation and apoptosis. While decellularized ECM can provide a natural niche for cell culture, it is important to identify the essential components in the ECM environment. So far, most approaches about investigating the interaction between cells and ECM were based on 2D cell culture which cannot fully mimic the *in vivo* state. Compared with 2D cell culture, 3D culture such as producing spheroid can better mimic the condition *in vivo*. The combinatorial screening of ECM component signals for 3D spheroid culture may improve our understanding of ECM effects on cell function. In this study, we developed an integrated approach to form 3D spheroid followed by encapsulation in hydrogel to systematically study the effect of different ECM components. Four ECM molecules (type I collagen, type IV collagen, laminin, fibronectin) effect on the maintenance of hepatocyte phenotype were evaluated in various 2D and 3D culture configurations, such as 2D monolayer culture and 3D spheroid encapsulated in hydrogel with defined ECM composition or non-encapsulated but supplemented with ECM molecules during cell aggregation process. Our results indicated the ECM molecules demonstrated distinct effects in 3D encapsulated spheroids of primary mouse hepatocyte, in which laminin showed an overall supportive effect compared with other ECM molecules. A combinatorial screening was performed to probe the cell response in a multitude of 3D microenvironments for identifying combinations of ECM that functioned synergistically. The findings of this study offered insight into optimizing ECM microenvironments for tissue engineering.

DRB9

The function of a primate-specific microRNA-1202 in dopaminergic neurons

Suyu HAO, Kai Kei MIU, Hoi Hung CHEUNG, Wai-Yee CHAN

Supervisor: Wai-Yee CHAN

Many miRNAs have been identified as biomarkers or molecular intermediaries in response to medical treatments. miR-1202 is also reported to be a significantly altered biomolecule through a number of profiling studies in various pathological conditions. As miR-1202 is conserved only in primates, little information was known about its molecular action and biological function from the commonly used laboratory animal models. miR-1202 was previously revealed being involved in major depression disorder (MDD). Our preliminary results, together with others, suggest that miR-1202 is brain enriched and tightly regulated during DA neuron differentiation.

Since we have established a method to generate DA neurons directly differentiated from iPSC, we followed this protocol to analyze the cellular phenotypes when miR-1202 is interrupted. Therefore, we generated miR-1202 knockout (KO) cells using CRISPR/Cas9 constructs and pick 1 clone with single copy. After generation of miR-1202 KO lines, iPSC (together with normal control) have been differentiated to DA neurons. We collected samples at day 0, 11, 27 which represent undifferentiated, committed progenitor and differentiated neurons, respectively. We compared the expression of markers for different regional identity and mid-brain DA (mDA) specification and neurogenesis between WT and KO cells.

We found a significant difference in the expression of mDA neuronal markers NURR1 and TH, the rate-limiting enzyme in the synthesis of dopamine. These two mDA neuronal markers were significantly decreased in miR-1202 KO cells, indicating impaired mDA neurogenesis. KO cells also showed much lower expression of TUJ1, a pan-neuronal marker, at Day 27. LMX1A, a homeobox transcription factor for the specification of mDA neurons, was not significantly changed until Day 27, KO cells expressed a slightly higher level of LMX1A. However, MSX1, a downstream target of LMX1A, which is known to promote mDA neuronal differentiation by inducing NGN2 and suppressing alternative ventral cell fates, was also significantly decreased in KO cells. Regional markers for ventral mid-brain such as OTX2 and FOXA2 were induced as early as at Day 11 and maintained at high level at Day 27. In contrast with mDA markers, OTX2 level was increased in KO cells at Day 11, whereas FOXA2 was consistently increased from Day 11 to 27.

DRB10

Bioengineering 3D Printed Auricular Scaffold for Microtia Treatment

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3D-printed, tissue engineered auricular scaffolds can enhance external ear reconstruction for treating congenital microtia. Current state-of-the-art techniques for auricular reconstruction involve harvesting costal cartilage and subsequent hand carving by a surgeon to produce an ear-shaped scaffold, which can introduce donor site morbidity and lengthen the operation duration, respectively. While patient-tailored ear scaffolds have been 3D-printed, integration of such scaffolds with an alternative, bioengineered source of chondrocytes stands to sidestep these challenges. Hence, we have initiated efforts both in developing the 3D printed scaffold and chondrogenic differentiation of muscle-derived cells to improve the auricular reconstruction.

To fabricate a framework for culturing bioengineered cartilage, we first employed 'click'-type chemistry to a 3D-print a novel biomaterial (abbreviated as PHT). Feasibility studies showed that an ear-shaped 3D scaffold approximately 36 mm by 20 mm could be printed at 300 μm resolution within several hours. To facilitate scaffold colonization, fibrin hydrogels were formed within the porous regions of 3D-printed PHT scaffolds. Pilot FTIR studies showed characteristic peaks of elastomer and fibrin within the same spectra, demonstrating that the successful integration of 3D-printed scaffold and hydrogel occurred. Next, to assess cytocompatibility and biological activity of 3D-printed ear scaffolds, we investigated spheroid cell viability and chondrogenesis using live/dead staining, qPCR, immunofluorescence staining, and cytochemical staining. Cell viability studies showed that there were almost no dead cells after seeding on scaffolds for 3 days, indicating no distinctly negative effect of PHT on cell viability. Cell differentiation studies showed that chondrogenic markers were upregulated at both gene and protein levels, including extracellular matrix. qPCR data showed that aggrecan and elastin were increased 25 and 429 folds, respectively ($p<0.05$), in the chondrogenic group relative to control. Similarly, immunofluorescence staining showed that Sox-9 and elastin were upregulated in the chondrogenic group relative to control. In addition, Alcian Blue staining showed that positive staining for glycosaminoglycans. Together, these preliminary results demonstrate that spheroid chondrogenesis differentiation was achieved. In summary, we have developed a 3D-printed auricular scaffold that can be combined with an alternative source of bioengineered chondrocytes. Our work holds great promise for avoiding costal cartilage donor site morbidity and shortening the duration of auricular reconstruction to improve outcomes for microtia patients.

Key words: 3D printing technology; biomaterial; scaffolds; auricular reconstruction.

DRB11

Non-collagen proteins are essential for the pro-tenogenic bioactivity of tendon extracellular matrix on human adipose stem cells

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Tendon healing is challenging, in part due to incompetent regenerative capacity and mechanically-demanding requirements. To aid tendon repair, we have developed a urea-based approach for preparing tendon ECM extracts (tECM), which exert remarkable pro-tenogenic bioactivity on human adipose stem cells (hASCs). The objective of current study is to identify the functional components of tECM (e.g. collagens and non-collagen components) to further elucidate its mechanisms of action, which is important to provide a rational basis for developing an ECM-based approach for functional tendon repair. In this study, hASCs and tECM was prepared and characterized as previously described [1]. To determine the role of tECM components on tenogenesis, tECM was treated by various enzymatic digestion (pepsin, chondroitinase, and hyaluronidase) and used as medium supplement for cell culture. Subsequently, hASCs were cultured with pepsin digested-tECM in combine with different tendon-associated growth factors (TGF-β1, TGF-β3, IGF-1, FGF-2) at 10, 50 ng/mL. The tenogenic differentiation effect on hASCs of different treated-tECM was determined using qPCR and immunostaining with tenogenic associated markers (tenascin C, type I collagen, scleraxis). Our results showed that pepsin which digested the non-collagenous components from tECM extracts significantly compromised the pro-tenogenic bioactivity of tECM, which could not be completely rescued by growth factor treatment. Thus, tECM bioactivity is partly mediated by non-collagenous components. Future work will seek to identify the specific non-collagenous components responsible for tenogenic bioactivity and elucidate their mechanistic actions as well as associated signaling pathways.

Key words: Non-collagen protein, Tendon ECM, Tenogenic effect

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DRB12

Thyroid hormone regulates heart regeneration in zebrafish

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Myocardial infarction (MI), also called a heart attack, commonly occurs when the coronary circulation is decreased or blocked at a part of the heart, which causes massive cardiomyocyte death or dysfunction and damage to the cardiac muscle, resulting in costly and potentially fatal conditions. Induction of post-MI cardiac regeneration has been a topic of intensive research in the field of regenerative medicine. Unlike adult mammals which repair damage by scar formation, zebrafish, some amphibians and neonatal mouse can fully regenerate its heart after injury. Thyroid hormone was implicated in regulating heart regeneration. However, the underlying mechanism is yet largely unknown. To elucidate the role of the thyroid hormone during heart regeneration, we used several methodologies such as real-time quantitative PCR and histochemical staining, to compare regenerative capacity of heart after apex resection in zebrafishes. We also treated the H9C2 cell line with thyroid hormone (T3) to attest the findings. Our results suggest that low level of thyroid hormone enhances cardiac regeneration, whereas high doses of thyroid hormone inhibits self-renewal of cardiomyocytes. These studies further our understanding on heart regeneration and shed light on the complexity during this process.

Key word: thyroid hormone, heart regeneration.

DRB13

The physiological functions of β-defensin 19 in the testis

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Supervisor: Ellis FOK

The β-defensin family possesses a broad-spectrum of antimicrobial activities. Extensive studies have demonstrated that some epididymal β-defensins modulate sperm functions and are indispensable for male fertility. However, the function of testicular β-defensins remains unexplored. β-defensins 19(Defb19) was highly expressed in stage VII-VIII of the seminiferous tubules. DEFB19 was chemotactically active towards spermatocytes but not spermatogonia. Overexpression of DEFB19 altered the proliferation, motility and adhesion ability of Sertoli cells. Further, *Defb19* knockout male mice were subfertile. Intriguingly, the level of DEFB119, the human homolog of Defb19, was higher in the infertile patients. Together, we have revealed the indispensable function of DEFB19/119 in the testis and male fertility.

DRB14

INDUCED REJUVENATION OF MESENCHYMAL STEM CELLS DERIVED FROM AGED HUMAN ADIPOSE TISSUE USING SMALL MOLECULE SC1231

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Supervisor: Kenneth K. H. Lee

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Human mesenchymal stem cells (MSCs) are multipotent connective tissue cells that are one of the most attractive stem cell source for tissue engineering and cell therapy. MSCs possess a potential to self-renew and differentiate into various types of cells such as osteoclasts, adipocytes, chondrocytes and neurons. Compared to the pluripotent stem cells these cells are also easier to harvest and free from ethical complications. MSCs have also good immunosuppressive capacity and they do not form teratomas in vivo. MSCs proliferate well in vitro but old patients' MSCs enter senescence a lot faster compared to the young patients' MSCs. This limits their use in tissue engineering and therapy since old patients are usually in higher demand of treatments. Therefore, it is crucial to enhance the proliferation capacity of these cells. In this project we tested an ability of the small molecule SC1231 to increase the proliferation of the aged adipose derived mesenchymal stem cells (AD-MSCs). Level of various proliferation markers such as phosphorylated histone H3 (PH3) and senescence associated β-galactosidase staining were observed. In addition, the protein or mRNA level of proliferation associated markers such as Ki67 and senescence associated markers such as p21 and p16 were investigated after the SC1231 treatment. Also the morphological changes were detected after the treatment. Furthermore, the expression of β-catenin and its downstream signalling genes were significantly elevated after SC1231 treatment. These results of this project have shown evidence that small molecule SC1231 has potential to re-activate the proliferation in aged AD-MSCs via Wnt/β-catenin signalling pathway.

DRB15

Improving oocyte quality through mitochondrial transfer from mesenchymal stem cells via tunneling nanotubes.

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Mitochondrion is a critical cellular factor that links to the poor reproductive outcomes in natural birth and *in vitro* fertilization (IVF) failure of aged women. Previous replacement therapies raise concerns on safety and flexibility. Recently we have successfully discovered a novel and natural method to transfer mitochondria to oocytes via tunneling nanotubes (TNTs) from MSCs. We demonstrated that mitochondria could pass through the zona pellucida of the mitochondrial dysfunctional oocytes. To reveal the effect of the transfer, we demonstrated that it can improve mitochondrial respiratory function and maturation rate of mitochondrial dysfunctional oocytes by 13.3% and 80.4% respectively. Importantly, we showed that the transfer efficiency was not limited by the age of MSCs. Mitochondrial transfer of aged MSCs increased aged oocyte maturation rate by 59.3% and fertilization rate by 50.0%. To delineate the genomic signatures induced by the transfer procedure, single-cell RNA-sequencing (scRNA-seq) was performed. Cluster analyses suggested the shift of genomic identity of *in vitro* matured (IVM) oocytes mimic the genomic identity of *in vivo* matured oocytes. RNA transcripts associated with common deletion region of mitochondrial genome and cyclic adenosine 3′,5′-monophosphate (cAMP) signaling were enriched after MSC mitochondrial transfer. In summary, we have found a cutting-edge solution for improving oocyte quality in the IVM procedure. The results provide a promising avenue that allows increasing the number of usable oocytes in clinical IVF.

DRB16

Induction and transplantation of neural crest cells from mouse induced pluripotent stem cells

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The enteric nervous system (ENS) is termed the “second brain” because of its ability to regulate the gastrointestinal functions in a complex and autonomous manner. Defects of the ENS development may lead to many congenital disorders including Hirschsprung’s disease (HSCR). HSCR is a common gastrointestinal motility disorder caused by absence or reduction of enteric neurons and/or their progenitors, namely neural crest cells (NCCs), in the distal colon. Cell therapy has been regarded as a potential treatment for this disorder. However, the cell sources for cell therapy have not been fully explored. Previously we established a three-step protocol to induce NCC formation from mouse induced pluripotent stem cells (miPSCs). The protocol includes sphere formation, NCC emigration and cell expansion. Here we further improved the NCC induction protocol by changing the cell seeding density and the duration of the emigration period. We then used a microsurgical method to transplant induced NCCs or spheres to the hindgut of embryos *ex vivo* or the distal colon of mouse *in vivo*. The results demonstrated that adding a ROCK inhibitor during the emigration period increased the efficiency of NCC induction by increasing the percentage of induced NCCs emigrated from the spheres. In addition, transplanted spheres were found to be able to survive both in the embryonic hindgut *ex vivo* and in the distal colon of mouse *in vivo* five days after the transplantation. Although the migration and differentiation of the transplanted NCCs in the hindgut *ex vivo* and in the distal colon *in vivo* require further investigations, our study have established a miPSC-based platform for studies of mouse NCC induction and gives insights into the feasibility of the stem cell-based therapy for HSCR.

DRB17

Growth factor-based, multi-functional regeneration for muscle and tendon in chronic massive rotator cuff tears

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Biological augmentation such as administering growth factors hold promise for treatment of clinically-challenging rotator cuff (RC) tears. Specifically, such approaches must not only target the bone-tendon interface, where tears frequently occur but also accompanying tendon degeneration/retraction and muscle fatty degeneration (FD), which are major contributing factors towards poor patient outcomes. In this study, we assessed combined use of Fibroblast Growth Factor-2 (FGF-2), Transforming Growth Factor-Beta3 (TGF-β3), and Insulin-like Growth Factor-1 (IGF-1) as a growth factor cocktail for simultaneous induction of tenogenesis and inhibition of adipogenesis to regenerate tendon and rescue FD, respectively. In tenogenic studies, human mesenchymal stem cells (hMSCs) were cultured with different combinations of FGF-2, TGF-β3 and IGF-1 under serum-containing and serum free conditions both in 2D and 3D (fibrin-based) microenvironments. First, our cocktail of FGF-2, TGF-β3, and IGF-1 promoted hMSC proliferation, demonstrated by increased DNA and Ki67 immunofluorescence staining. Second, this growth factor cocktail also increased hMSC tenocyte differentiation, demonstrated by higher expression of tendon-associated markers *Scx*, *Tnc*, and *Col1a1* at gene and protein levels, promoted extracellular matrix deposition of Tnc synthesis and collagen. Third, fibrin-based 3D constructs incorporating both mechanical tension and our growth factor cocktail promoted collagen synthesis. In muscle FD studies, *in vitro* and *in vivo* models were generated to mimic FD and assess the effect of our GF cocktail on minimizing adipogenesis. In our *in vitro* studies, C2C12 myoblast cells and human muscle-derived stem cells (hMSDCs) independently cultured in a mixture of myogenic and adipogenic media showed significant reduction in Oil-Red-O stained lipid droplet accumulation when treated with FGF-2, TGF-β3 and IGF-1, indicating successful inhibition of adipogenic differentiation. In our *in vivo* studies, chronic four-week muscle-tendon segmental injuries were created in rabbit rotator cuff tissue (infraspinatus + supraspinatus or subscapularis alone). Histological comparison of muscle-tendon injury induced at different rotator cuff sites established the subscapularis injury site as exhibiting the most severe fibrosis and FD model in rabbit and will be used in future GF cocktail evaluation. Collectively, these findings demonstrate combined use of FGF-2, TGF-β3, and IGF-1 as a promising strategy for simultaneous induction of tenogenesis and inhibition of adipogenesis, paving the way for multi-tissue, rotator cuff regeneration.

Key words: Tenogenesis, serum-free medium, Growth factor, fatty degeneration, rotator cuff, musculoskeletal tissue engineering.

DRB18

Mechanistic study of transcription factor SOX18 during heart development

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Heart development is a delicate and complex process involving massive migration and differentiation of heart precursor cells under the regulation of various signaling pathways. The Sox family consists of a group of transcriptional regulators defined by the presence of a highly conserved high-mobility group (HMG) domain that mediates DNA binding, regulating a broad range of biological processes including cell fate determination and cell lineage differentiation. Among the SOX family, the Sox F group has been implicated in the vasculogenesis, cardiogenesis and lymphangiogenesis. Mutations in this gene group are correlated significantly with the etiology of human vascular disease in a redundant manner. Sox18 belongs to the Sox F group, and it contains transcriptional activation domain (TAD) and SRY-related DNA-binding HMG-box and a short amino acid motif (DXXEFD/EQYL) inside the TAD. The amino acid motif was reported to mediate the interaction with β-catenin, a key regulator of Wnt signaling pathway. We found that *sox18* is expressed in heart, branchial arch, pharyngeal arch, spinal cord, and intersegmental vessels during the tailbud stage of *Xenopus tropicalis*. Both gain-of-function and loss-of-function of *sox18* in the *X. tropicalis* embryos cause decreased expression of *gata4* and *myh6*, suggesting a proper *sox18* expression is required for heart development. β-catenin is the key regulator of the Wnt signaling pathway that is essential for cardiogenesis. TOPFLASH shows that *sox18* can suppress the activation of Wnt reporter genes, suggesting a suppressive role of Sox18 on β-catenin/Wnt signaling pathway. In our future study, we will investigate the underlying mechanism as to how Sox18 regulates the Wnt signaling pathway and elucidate its role during cardiogenesis.

Key words: *Xenopus*; heart development; Sox18; Wnt signal pathway; transcriptional factor.

The Potential Role of RSPO3 in Oligodendrogenesis

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Backgrounds:

Oligodendrocytes play significant role in providing support and insulation to axons via myelination in the central nervous system (CNS). The malfunction of oligodendrogenesis or myelin loss results in neurological disorders, such as multiple sclerosis, leukodystrophies, etc. However, the molecular mechanisms underlying oligodendrogenesis and myelination in CNS development and diseases are still elusive. The R-spondin (RSPO) family of four proteins represents a new group of secreted factors that enhance β-catenin signaling. Like WNTs, RSPOs have important roles in development and act as powerful stem cell growth factors. In this study, we aim to investigate the role of R-Spondin3 (RSPO3) in Oligodendrogenesis.

Results:

We found that the expression of RSPO3 increased along with postnatal development. RSPO3 was expressed mainly at hippocampus, subventricular zone, corpus callosum, striatum and cerebellum at postnatal stage (PN1-PN30) in mice. Of note, the expression of RSPO3 emerged at postnatal day 7 and peaked at postnatal day 30, overlaying with oligodendrocyte lineage markers, O4 and MBP. These results indicate a potential role of RSPO3 in oligodendrogenesis. In line with this, the *in vitro* differentiation assay of neural progenitor cells (NPC) showed that RSPO3 increased along with the oligodendrocyte differentiation. Moreover, addition of recombinant RSPO3 in NPC enhanced the oligodendrocyte differentiation whereas had no effect on neurons or glia. We also isolated primary OPC from newborn mice and rat and found that RSPO3 enhanced the proliferation and differentiation potentiation of the OPC, but had no effect on neurons or mesenchymal stem cells.

Conclusion:

Together, these data indicate that RSPO3 may act as a positive regulator of oligodendrocyte development. This study will not only reveal previously undefined role of RSPO3 in oligodendrogenesis and brain development, but also provide important insights into the underlying mechanism and potential therapeutic target of demyelinating diseases.

Key words: R-spondin 3, oligodendrocyte, demyelinating diseases.

Identification of essential factors during *Xenopus* heart regeneration

Lin Yi-jyun, Zhao Hui

Supervisor: Zhao Hui

Cardiovascular diseases commonly caused myocardial damage. However, the mammal heart has limited ability on regeneration. *X. tropicalis* is an amphibian species that phylogenetically closer to mammals but still remains its well regeneration ability in heart. A heart regeneration model was setup in order to elucidate the mechanism as to how the *X. tropicalis* regenerated its heart after heart apical resection. The isobaric tags for relative and absolute quantitation (iTRAQ) mass spectrometry technique was used to investigate the underlying mechanism of *Xenopus* heart regeneration. Furthermore, H&E staining showed that the *X. tropicalis* heart could be repaired after 60 days from surgery. The top 15 pathways from day 1 to day 3 after surgery samples involved pathways such as neutrophil degranulation and apoptosis. The 4th to 16th days' pathway analysis indicated that the immune responses were reduced and translation related molecular activity become abundant. In summary, the mass spectrometry results provided an insight into the protein alternation after *Xenopus* heart surgery. This project will further focus on whether cardiomyocyte proliferation and a novel target, XENTR_v90029515mg protein, contribute to the heart regeneration ability in *Xenopus* based on the mass spectrometry results.

Key words: heart regeneration, *Xenopus*, iTRAQ

Developing a molecular age model for oocyte assessment

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In vitro fertilization (IVF) is generally presented as a viable option but the success rate is limited by maternal age. The low success rate is also compound by subjective visual quality assessment, which creates a huge challenge for AMA women. A novel genetic-based oocyte assessment platform for age prediction using mouse oocytes had been developed in this study. Based on the transcription profile of both immature (GV) and mature (MII) oocytes in young (6 weeks old) and aged group (12 months old) obtained by single cell RNA sequencing, we were able to identify the differential expression genes (DEGs) that govern the aging process. A total of 1377 DEGs in GV oocyte aging were identified. Of which 745 genes were up- regulated and 631 genes were down-regulated. On the other hand, we compared the transcriptome of MII oocytes and identified 1432 DEGs, 706 of them were upregulated and 726 were downregulated DEGs in aging process. The candidate genes for the age prediction model was selected by logistic regression model classifier with L1 norm penalty from the above sample, the power of prediction was ROC=0.81. Furthermore, we are developing a scoring system of 1-10 to give a clear grading regarding the oocyte quality.

DRB22

The role of KDM3A in vascular function and regeneration: implication of aging

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KDM3A, also known as JMJD1A or JHDM2A, has the specific ability to demethylate H3K9me1 and H3K9me2. Our previous research demonstrates for the first time that it is negatively correlated to human stem cell aging, and that loss of it leads to bone aging. In order to thoroughly investigate the role of KDM3A in aging, we extend our research interest to vascular function and regeneration since vascular aging contributes to geriatric diseases, such as stroke, heart attack, dementia, sarcopenia, and osteoporosis. In this project, we found that KDM3A was decreased both in aorta from older mice and in older passage of primary murine endothelial cells (MLECs). Furthermore, we found that depletion of KDM3A resulted in vascular dysfunction, including increased adventitia to media ration, vascular smooth muscle cell loss, and more breaks per section in KO aorta. Mouse hind limb ischemia also demonstrated that KDM3A might play an important role in vascular regeneration as angiogenic defect was determined both by Laser Doppler Perfusion Imager and immunostaining of CD31. Last, we found that KDM3A inhibition resulted in angiogenic defects and KDM3A over-expression promoted angiogenesis in HUVECs. Taken together, these findings suggest that KDM3A regulates endothelial cell function, defect of which leads to defect in vascular repair and vascular aging.

Key words: KDM3A, vascular aging, endothelial cells

DRB23

Single cell ATAC-Seq reveals epigenetic heterogeneity associated with an EMT-like process in male germline stem cells

Hoi Ching SUEN*, Jinyue LIAO*, Chun Shui LUK, Wing Tung LEE, Judy Kin Wing NG, Ting Hei Thomas CHAN, Man Yee CHEUNG, Robin HOBBS[#], Tin-Lap LEE[#]

Supervisor: Tin-Lap LE

Epithelial-mesenchymal transition (EMT) is a phenomenon in which epithelial cells acquire mesenchymal traits. It contributes to organogenesis and tissue homeostasis, as well as stem cell differentiation. Emerging evidence indicates that heterogeneous expression of EMT gene markers presents in sub-populations of germline stem cells (GSCs). However, the functional implications of such heterogeneity are largely elusive. Here, we unravelled an EMT-like process in GSCs by *in vitro* extracellular matrix (ECM) model and single-cell genomics approaches. Through modulating ECM, we demonstrated that GSCs exist in interconvertible epithelial-like and mesenchymal-like cell states. GSCs gained higher migratory ability after transition to a mesenchymal-like cell state, which was largely mediated by the TGF-β signaling pathway. Dynamics of epigenetic regulation at the single-cell level was also found to align with the EMT-like process. Chromatin accessibility profiles generated by single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) clustered GSCs into epithelial-like and mesenchymal-like states, which were associated with differentiation status. The high-resolution data revealed novel regulators in the EMT-like process, including transcription factors *Zeb1* and *Hes1*. We further identified putative enhancer-promoter interactions and *cis*-co-accessibility networks at loci such as *Tgfb1*, *Notch1* and *Lin28a*. Importantly, we demonstrated that histone methyltransferase G9a promoted the GSC EMT-like process *in vitro* and contributed to neonatal germ cell migration *in vivo*. Our work thus provides the foundation for understanding the EMT-like process and a comprehensive resource for future investigation of epigenetic regulatory networks in GSCs.

DRB24

A Physiological Dynamic Mechanical Loading Platform for Meniscus Tissue Engineering

SUN Jing, CHAN Yau Tsz, HO Ki Wai Kevin, TUAN Sung Chi Rocky, JIANG Yangzi

Supervisor: TUAN Sung Chi Rocky, JIANG Yangzi

Meniscus tears are frequently encountered in clinical practice, and while partial or complete meniscectomy is a common treatment option, general meniscus loss is a risk factor for the development of osteoarthritis. Efforts are made to achieve seamless healing of meniscus tears, mainly involving the usage of biocompatible hydrogel and tissue specific stem/progenitor cells. As a load-bearing tissue, the dynamics of meniscus repair in vivo are governed by both of biological and biomechanical cues. To mimic authentic load-bearing meniscus, dynamic mechanical stimulation is indispensable, which can recapitulate the mechanical microenvironment of meniscus, regulate the release profile of the preselected growth factors, and promote the meniscus healing process. We here have designed and developed a homemade hydrogel-based bioreactor, which can provide repetitive mechanical loading to 3-dementenal (3D) cultured meniscus progenitor cells, thus mimic the mechanical environment of meniscus healing in vitro.

Mechanical sensitive tissue progenitor cells, the human meniscus stem/progenitor cells (hMeSPCs), were isolated, expanded and characterized in vitro, and encapsulated in Gelatin Methacryloyl (GelMA) hydrogel. Controlled tensile strain was applied to the hydrogel-encapsulated cells through the homemade 3D bioreactor, which enabling the 3D-cell culture of hMeSPCs for more than 15 days with 94% of the cell survival rate. We found that hMeSPCs displayed mesenchymal stem cell characteristics after isolation, and the controlled tensile strain loading enhanced the differentiation of GelMA encapsulated hMeSPCs, compared with the static group. The re-establishment of the extracellular matrix (ECM) under tensile strain loading was increased. Furthermore, less cell senescence was observed after tensile treatment, compared with the static control group. These findings show that mechanical loading assists meniscus derived progenitor cell differentiation, preventing cell senescence, and it is a promising advanced platform for deciphering the biomechanics in both cell and tissue levels.

DRB25

NAD+ Supplement Rescues Adipogenesis and Metabolism in Werner Syndrome

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Werner Syndrome (WS), also called progeria, is a hereditary condition associated with premature aging and an increased risk of cancer and other diseases. Signs of Werner Syndrome usually develop in the childhood or teenage years, which usually includes type 2 diabetes, dysregulation of metabolism, gray hair and hair loss, etc. Metabolic dysfunction is a primary feature of WS, who exhibits severe metabolic deficit. We generated the WRN-/- stem cell model using Crispr/cas9 technique. Our current work found that the loss of WRN could cause dysregulation of metabolism in vitro. The adipogenesis was prematured in WRN-/- cell model compared with the wildtype, and some adipocyte markers, CEBP and PPAR alpha, which were supposed to express on day 14 or day 21 showed up much earlier. However, when NAD+ was replenished, the adipogenesis could be rescued. Also the zebrafish model system showed similar phenomenon, which might help explain why Werner Syndrome patients had metabolic dysfunction. Then we did transcriptomic RNA-Seq and found out the loss of WRN during adipocyte development might caused by mitophagy. Our results demonstrated that NAD+ augmentation restored mitochondrial biogenesis and adipogenetic metabolism both in vitro and in vivo.

DRB26

In vitro 3D liver model utilizing iPSC induced hepatocytes for drug testing

Tsang Hoi Ying, Lee Ka Ho Kenneth

Supervisor: Lee Ka Ho Kenneth

Liver is responsible for the detoxification in our bodies, and it is exposed to different threats in everyday life. Despite of the strong regenerating property of the organ, when the exposure of toxic substances is prolonged and beyond its limits, the damage could be irreversible, as the extracellular matrix proteins which are supposed to heal wound over accumulates, forming extensive scar tissues in the liver. Indeed, chronic liver diseases including liver cirrhosis have been one of the highest leading causes of death in Hong Kong and in other developed countries for these decades, yet no cures are found as the drug discovery process relies on the *in vitro* cell model growing on petri dishes, which is lack of representativeness to their *in vivo* counterparts, on the other hand animal models are found not predictive for the human responses to drug. Our study aims at creating a novel 3D *in vitro* functional liver model by co-culturing induced hepatocytes with non-parenchymal liver cells including hepatic stellate cells, endothelial cells as well as mesenchymal stem cells, all derived from human induced pluripotent stem cells (iPSCs) from the same donor. We successfully generated vascularized liver organoids that secret albumin, produce urea and exhibit many other liver functions. In vivo liver microstructures, such as bile canaculi, were also present in the organoids, which proved that 3D co-culturing of the four types of cells better mimicked human microenvironment with enhanced cell-cell/cell-ECM interactions and allowed more accurate drug screening results. We believe that this study using iPSC induced hepatocytes gives novel insight in development of personalized medicine, increasing the effectiveness of pre-clinical studies and reducing the number of experimental animals needed in drug discovery process.

DRB27

Development of Gene Therapy for β-Thalassaemias through Therapeutic Genome Editing in Human Blood Stem Cells

URIP Brian Anugerah, Feng Bo

Supervisor: Feng Bo

β-thalassaemias are highly prevalent inherited blood disorders caused by deficiency or absence of the β-globin subunit of adult hemoglobin. Patients with severe form of β-thalassaemias (β-thalassaemia major) need to receive life-long regular blood transfusions, usually every two to five weeks. Frequent blood transfusions might lead to several complications like iron overload and infections which pose serious burdens and risks to the patients' health. In this project, we are utilizing the ground-breaking CRISPR/Cas9 technology, coupled with the clinically approved gene delivery vector, recombinant adeno-associated virus (rAAV). The system is aimed to employ a gene knock-in delivering a functional β-globin (HBB) gene into patients' hematopoietic stem cells (HSC) and examine the potential of these cells in reconstituting healthy hematopoietic system and correcting the disease phenotype. Using K562 cells, an erythroleukemic cell line that has been extensively used for globin gene expression study, we observed that the knock-in efficiency at through homology directed repair (HDR) is superior to the non-homologous end joining (NHEJ) pathway. In order to identify the suitable locus for gene knock-in, the K562 cells are triggered to undergo erythroid differentiation and the expression of various globin genes are examined. The knock-in efficiency in these loci and the protein level of the functional β-globin will be analysed throughout the erythroid differentiation process of K562 cells.

DRB28

Development of an in vitro hematopoietic stem cell niche on-a-chip

WAN Ho Ying, Anna BLOCKI

Supervisor: Anna BLOCKI

Hematopoietic stem cells (HSCs) give rise to all blood cells (hematopoiesis) and reside in the so called "bone marrow niche". The bone marrow niche is essential to maintain stemness of HSCs. Upon initiation of differentiation, they will leave the bone marrow niche and enter the circulation. There are two compartments in bone marrow niche, namely the vascular and endosteal compartment. HSCs require both compartments to maintain their stemness.

Engineering of the bone marrow niche has the potential to provide a platform to investigate the physiology of HSCs like self-renewal, proliferation and differentiation, as well as pathophysiological processes such as leukaemia. Further, it could be utilized as a drug screen platform for HSC-related diseases. However, past approaches using conventional cell culture technology, like 2D and 3D culturing, could not mimick successfully. Thus, more advanced approaches to build the bone marrow niche in microfluidic devices were proposed.

In this project, we aim to build a functional human bone marrow niche in vitro in a microfluidic device, which enables us to include a stable perfusable microvascular network (resembling the vascular compartment) and bone-like surface (resembling the endosteal compartment) in the microfluidic device. Our current work focuses on establishing both compartments separately. Upon confirmation of their functionality, both compartments will be integrated and investigated for their potential to maintain HSCs and hematopoiesis comparable to physiological conditions.

DRB29

Comparison of migration and differentiation of enteric neural crest cells and hair follicle-derived neural crest stem cell both in the hindgut explant cultured *ex vivo* and in the distal colon of adult mouse *in vivo*

Y. Zhang, T. D. Huang, C. M. Foo, W. Y. Chan

Supervisor: W. Y. Chan

School of Biomedical Sciences, The Chinese University of Hong Kong

Background: Hirschsprung's (HSCR) disease is characterized by absence of ganglia in the distal bowel. Enteric neural crest cells (ENCCs) have been demonstrated as one of the most effective cell sources to aid the regeneration of missing ganglia of the enteric nervous system (ENS) in the cell therapy. However, the limited availability of such cell type hinders the further development of the cell therapy for HSCR. In the present study, we characterized a new type of stem cells which were isolated from hair follicles and named hair follicle-derived neural crest stem cells (HF-NCSCs). They can potentially serve as a source of stem cells in the cell therapy for HSCR.

Method: ENCCs were isolated from the hindgut of early postnatal mouse, and HF-NCSCs were obtained from mouse hair follicles. Both ENCCs and HF-NCSCs were cultured as neurospheres in a serum-free culture medium. Expression of neural crest stem cell markers were determined by immunofluorescence staining. The migration and differentiation potential of ENCCs and HF-NCSCs were further evaluated by cell transplantation to both the hindgut explant cultured *ex vivo* and the distal colon of adult mouse *in vivo*.

Results: Both ENCCs and HF-NCSCs were found to be able to express neural crest stem cell markers nestin, Sox10 and p75. Differentiation assays in different differentiation media showed their potentials to form Tuj1-positive neurons and GFAP-positive glial cells. Five and six days after they were transplanted into hindgut explants from E11.5-E12.5 mouse embryos, cells of the transplanted ENCCs and HF-NCSCs neurospheres spread along the gut explant and formed Tuj1-positive fibers. Seven and fourteen days after they were transplanted by microinjection into the distal colon of adult mouse, both ENCCs and HF-NCSCs neurospheres survived and extended fibers from the transplantation site.

Conclusion: Our results support the feasibility of using HF-NCSCs in the cell transplantation as a viable therapeutic option for HSCR.

DRB30

CRISPR/Cas9 mediated knock-in for the treatment of inherited hemophilia

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Supervisor: FENG Bo

(School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, SAR, China)

Hemophilia A and B are X-linked bleeding disorders caused by mutations in genes encoding the coagulation factors VIII and IX (*F8* and *F9*) respectively. Adeno-associated virus (AAV) based gene therapy to deliver B domain-deleted *F8* (*BDDF8*) for hemophilia A treatment has already entered the clinic and showed great progress. However, in cases of pediatric patients, the persistency of AAV genome is limited owing to the high rate of cell turnover in young patients. Hence, we focus on AAV-mediated therapeutic genes knock-in through CRISPR/Cas9 technology to permanently insert *BDDF8* gene into the defected host genome for hemophilia A treatment. Another emerging issue is the formation of inhibitory antibodies (20%-30% in severe hemophilia A; 5% in severe hemophilia B), which remains as a critical challenge to the current treatment methods. Recombinant FVII (F7) has been used as a bypassing agent to solve the problem. It was shown that sustained exogenous expression of *F7* gene mediated by AAV has the potential to correct hemophilic conditions even in the presence of inhibitory antibodies to FVIII. We hypothesize that AAV-mediated genes knock-in of F8 and F7, combined with CRISPR/Cas9 technology is a promising strategy for the treatment of inherited hemophilia. Further analyses will be carried out to evaluate the knock-in efficiency and therapeutic effect in hemophilia mice model.

DRB31

Novel Intrinsic and Extrinsic Regulation of Spermatogonial Stem Cells Maintenance

Zheng Tingting, Ellis Fok

Supervisor: Ellis Fok

Spermatogenesis, a highly complex and regulated process, relies on a delicate balance between self-renewal and differentiation of spermatogonial sperm cells (SSCs). Many factors affect SSCs maintenance, which can be divided into intrinsic and extrinsic factors according to whether produced by SSCs.

Here, the ubiquitin ligase Huwe1, has been proved to be one of these intrinsic factors in our previous study. Although the inactivation of Huwe1 causes apoptosis in many types of cells, we find HUWE1 knockout in C18-4 cell line arrests spermatogenesis not by apoptosis. To further study the possible regulatory mechanism behind, the proteomics technology is applied here to find out affected proteins after HUWE1 knockdown. Totally, preliminary 212 differentially expressed proteins are identified. Further candidates selection and validation would be conducted accordingly to illustrate the intrinsic pathways related.

Besides, extracellular vesicles (EVs) carry cargoes including DNAs, RNAs, proteins and lipids, which play roles in multiple biological processes. Here, we find SSCs can uptake testicular extracellular vesicles (tEVs), and spermatogenesis is somehow affected. Apparently, some cargoes of tEVs may participate in SSCs maintenance and thus belong to extrinsic regulation. Further identification of the functional components of these extrinsic factors by proteomics and transcriptomics and illustrating the mechanism behind would be the other part of our work.

DRB32

Functional characterization of a hydrogel-based bioink for fabrication of engineered cartilage like tissue

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Supervisor: Chao Wan

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong.

Articular cartilage, a hyaline cartilage covering the articular surfaces of bones, provides support to human joint movement and weight bearing due to its unique structural and mechanical properties. Being an avascular and aneural connective tissue, articular cartilage has very limited capacity of self-repair following injury or degeneration. Cartilage tissue engineering has become a promising approach for facilitating articular cartilage repair or generating cartilage tissue models for drug testing. Integrating chondrogenic cells, bioactive molecules and printable biomaterials, 3D bioprinting has emerged as a novel fabrication method for automated control of the architecture of the articular cartilage tissue. However, the bioink that can be used for 3D bioprinting of cartilage like tissue remain limited. In this study, we generated a type of hydrogel-based bioink incorporating with chondrogenic cells for fabrication of engineered cartilage like tissue. The hydrogel-based bioink was composed of alginate or alginate/gelatin compound laden with mouse chondrocytes. The hydrogel-based bioink was shown to possess excellent printability, biocompatibility and structural fidelity. In the fabricated 3D constructs, above 98% of chondrocytes were maintained in the cultures at days 1, 5 and 7 following the extrusion-based 3D bioprinting. During the up to 21 days cultures of the 3D bioprinted constructs, the chondrocytes maintained chondrogenic phenotypes as indicated by expression of chondrogenic marker genes by real-time PCR, the production of cartilage extracellular matrix (ECM) components including proteoglycan indexed by Safranin O staining and collagen type II shown by immunostaining in the tissue sections of the 3D constructs. In addition, the bioink composed of alginate/gelatin compound hydrogel exhibited improved structural fidelity following fabrication compared with that of the alginate hydrogel. Our results established a guideline for generating hydrogel-based bioink for fabrication of engineered cartilage like tissue that can be used for further *in vivo* testing.

DRB33

Modulation of macrophages by bioactive glass/sodium alginate hydrogel is crucial in skin regeneration enhancement

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Inflammatory response is a critical stage in typical wound healing. Although studies have reported that some bioactive materials can modulate the polarization of macrophages to benefit tissue regeneration, the roles of the inflammatory responses, especially the crucial roles of macrophages, in tissue regeneration stimulated by bio- materials remains unclear. Bioactive glass (BG) and hydrogel containing BG have been reported to be able to promote both hard and soft tissue regeneration. However, the critical roles of macrophages in tissue regeneration enhanced by BG have not been fully elucidated. In this study, the effects of BG/sodium alginate (SA) hydrogel (BG/SA hydrogel) on the behaviors of macrophages as well as on the interactions between macrophages and repairing cells were investigated. In addition, macrophage-depleted mice were used to investigate the necessity of macrophages in the regeneration of full-thickness skin wounds treated with BG/SA hydrogel. Our results indicated that BG/SA hydrogel could polarize macrophages towards M2 phenotype in vitro and in vivo and upregulate the expression of anti-inflammatory genes. In addition, the M2 polarized macrophages could further recruit fibroblasts and endothelial cells as well as enhance the extracellular matrix (ECM) synthesis of fibroblasts and vascularization of endothelial cells in vitro and in vivo. Depletion of macrophages in the wound sites impeded the recruitment of repairing cells and reduced the formation of blood vessels and ECM, slowing down skin regeneration. These results provide an insight into the biomaterial-immune system interactions and demonstrate that modulation of macrophages by BG/SA hydrogel in the inflammatory response is crucial in skin regeneration enhanced by the hydrogel.

DRB34

Microfluidic chips as a 3-dimensional experimental tool for studies of single cell migration

ZHU Yu, HO Yi Ping Megan, BLOCKI Anna Maria and CHAN Wood Yee

Supervisor: CHAN Wood Yee

Hirschsprung's disease is one of the most common congenital gastrointestinal motility disorders in newborns, which is caused mainly by deficiency of enteric neurons derived from neural crest cells. It has been suggested that the abnormal neural crest cell migration during the early embryonic development may be one of the possible causes of the disease. Previous studies on the migration of neural crest cells have made use of 2-dimensional (2D) cell culture, *ex vivo* gut explant culture and *in vivo* cell transplantation. However, the cell migration in a 2D system may deviate from the observations made under 3D conditions, whereas *ex vivo* gut explant culture can hardly be used for studies of single cell migration, and cell migration in living animals is very difficult to be monitored *in vivo*. To circumvent these difficulties, we used microfluidic chips to study cell migration in the present study. This microfluidic system provided a 3D environment composed of hydrogel of the enteric extracellular matrix (ECM) for cell migration. Additionally, chemotactic agents could be used in the system for the investigations of their possible roles in cell migration. The ECM was extracted from the whole intestinal tract of E18.5 mouse embryos with hypotonic and hypertonic solutions. To isolate enteric neural crest cells (ENCCs), midgut segments from mouse embryos at E12.5 were explanted and cultured *in vitro*. One day after the explant culture, ENCCs emigrated from the midgut explants were collected for cell migration studies. ECM Hydrogel was made of type I collagen, fibronectin and laminin, all of which are also found in the gut wall through which ENCCs migrate during the early embryonic development. Microfluidic chips were designed for single cell migration studies and prepared with PDMS which was treated with plasma to make its surfaces more compatible with cell culture. The three channels of the microfluidic chip were used separately for loading cells, hydrogel and chemotactic agents. Two days after loaded to the microfluidic chip, ENCCs were able to survive in the hydrogel and showed migratory morphologies similar to those of migratory cells in the developing gut explant *ex vivo* but different from those observed in 2D cell culture *in vitro*. Our findings indicate that the microfluidic chip is an appropriate and a useful experimental tool for studies of single cell migration.

NEURAL, VASCULAR AND METABOLIC BIOLOGY

Neural, Vascular, and Metabolic biology

Title	Author	Abstract Number
Endothelial SIRT3 mitigates atherosclerosis through regulating mitochondrial protein acetylation	CAO Xiaoyun	NVM1
Endothelial cell Nrf2 activation inhibits atherosclerosis	HE Lei	NVM2
Myeloid Bmal1 deletion prevents mice from house dust mite-mediated lung allergy	HONG Huiling	NVM3
Repurposing Thioridazine for Anti-atherosclerotic Treatment	JIANG Minchun,	NVM4
PKD2-associated cardiomyopathies in 3D engineered cardiac tissue model.	LI Jingxuan	NVM5
Role of TRPC5 in endothelium-dependent contraction of hypertension mice model	LI Xiao	NVM6
PPARα activation inhibits endothelial cell inflammation and atherogenesis	PU Yujie	NVM7
Exploiting the Random Forest classification algorithm for identifying features of muscle synergy post-stroke	SO Lester Kam Pang	NVM8
The Role of Endothelial PPARδ in Vascular Homeostasis	WU Yalan	NVM9
Mapping motor output units encoded in the human primary motor cortex with transcranial magnetic stimulation	XIE Jingping	NVM10
Regulation of Orai1 in Angiotensin II-Induced Cardiac Hypertrophy	XIE Mingxu	NVM11
A Potential Crosstalk between YKL-40 and PTEN In Amyloid Beta Induced Signalling and Biological Effects in Alzheimer's Disease	ZENG Xiaoyan	NVM12

NVMB1

Endothelial SIRT3 mitigates atherosclerosis through regulating mitochondrial protein acetylation

CAO Xiaoyun, Tian Xiaoyu

Supervisor: Tian Xiaoyu

Atherosclerosis is the inflammation of vascular wall triggered by initial dysfunction of the luminal endothelial cells potentiated by high cholesterol which leads to immune cell accumulation underneath the endothelium, forming atherosclerotic plaques. Sirtuin 3 (SIRT3) is a nicotinamide adenine dinucleotide (NAD+) -dependent deacetylase, mainly functioning in the mitochondria to regulate protein acetylation, which plays a key role in maintaining normal mitochondrial function via its anti-oxidative, anti-aging, and anti-inflammatory effects. The present study tested whether endothelial-selective Sirt3 deletion accelerates endothelial inflammation and atherosclerosis through hyperacetylation of mitochondrial proteins. To test the hypothesis, atherosclerosis was induced by feeding endothelial-specific Sirt3 knockout and wild type mice after PCSK9 overexpression, or Apoe knockout mice, with high cholesterol diet for 3 months. It was found that selective deletion of Sirt3 in endothelial cells exacerbates vascular inflammation, vascular dysfunction and plaque formation during the development of atherosclerosis. Meanwhile, NAD+ precursor nicotinamide riboside supplement which increased Sirt3 activity inhibited mitochondrial oxidative stress, diminished vascular inflammation and reduced plaque formation. In mouse endothelial cells, Sirt3 knockdown by shRNA exacerbates TNF-α-induced up-regulation of pro-inflammatory cytokines, Sirt3 knockdown also reduced mitochondrial respiration in endothelial cells. These data support the role of mitochondrial Sirt3 in endothelial dysfunction and atherosclerosis which will provide insight into endothelial mitochondrial dynamics regulated by post-translational modification in vascular inflammation of atherosclerosis. Further study is needed to identify protein target of SIRT3 in mitochondria which affects endothelial cell function.

NVMB2

Endothelial cell Nrf2 activation inhibits atherosclerosis

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Supervisor: Yu Huang

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Background: Atherosclerosis is a chronic inflammatory disease that is closely associated with angina, stroke, and aneurysm. Endothelial cell (EC) dysfunction caused by inflammation and oxidative stress is critical for the initiation and progression of atherosclerosis. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a transcriptional factor, is one of the most important players that limit oxidative stress and minimize the expression of inflammatory genes. However, whether endothelial Nrf2 can inhibit atherogenesis is still unknown.
Objective: To explore whether and how EC-specific Nrf2 activation impacts the development of atherosclerosis in athero-prone mice.
Methods and Results: Immunofluorescence data showed an elevated level of 4-hydroxynonenal in endothelial cells of atherosclerotic aortas, suggesting that lipid peroxidation in ECs increased during progression of atherosclerosis. The qPCR results showed that the expression of Nrf2 target genes (e.g. heme oxygenase-1 and NAD(P)H dehydrogenase 1) were upregulated in aortic ECs from *ApoE*$^{-/-}$ mice after 8- and 16-week feeding of western diet. To validate the role of endothelial Nrf2 in atherogenesis, we knocked down Nrf2 in *ApoE*$^{-/-}$ mice using CRISPR-Cas9-mediated gene editing technique and demonstrated that EC-specific knockdown of Nrf2 facilitated progression of atherosclerosis. Likewise, treatment of brusatol, a Nrf2 inhibitor, attenuated partial ligationinduced plague formation in the carotid arteries of *ApoE*$^{-/-}$ mice. Either *in vitro* knockdown of Nrf2 or treatment with Nrf2 inhibitor increased the expression of tumor necrosis factor α (TNF-α) induced inflammatory genes. By contrast, Nrf2 activation by dimethyl itaconate or through knocking down Kelch-like ECH-associated protein 1(Keap1) suppressed TNF-α-induced EC inflammation and monocyte attachment to ECs.
Conclusions: The present results indicate that endothelial Nrf2 is athero-protective, probably through its anti-inflammatory effects related to atherogenesis. Targeting endothelial Nrf2 could become a promising strategy for prevention and treatment of atherosclerotic vascular diseases. [This work is supported by Hong Kong Research Grants Council - General Research Fund (No. 14109720, 14112919)]

NVMB3

Myeloid Bmal1 deletion prevents mice from house dust mite-mediated lung allergy

Hong Huiling, Tian Xiaoyu

Supervisor: Tian Xiaoyu

Asthma is a chronic inflammatory airway disease that is characterized by airway obstruction, persistent lung inflammation, and bronchial hyper-responsiveness to inhaled stimuli. One of the most common allergens inducing lung asthmatic reactions is the house dust mite (HDM), which activates type 2 immune responses, leading to pulmonary eosinophils influx and the HDM-specific IgE production. The circadian rhythm in pulmonary physiology and pathology is long appreciated that both the respiratory function and the asthmatic responses show day-and-night variance. However, the molecular mechanisms behind the temporal control are poorly understood. To investigate the circadian regulation of HDM-induced allergic inflammation in the lung, we studied the role of the core clock gene Bmal1 in macrophages in a chronic HDM-challenging mouse model. We first explored the temporal variation of the lung inflammation by comparing the early rest phase ZT2 and the early active phase ZT12. We showed that more eosinophils infiltrated into the airspace and more Th2 cell differentiation in the lung-draining lymph node at ZT2 than ZT12. Myeloid deletion of Bmal1 abolished this day-and-night difference, indicating a regulatory role of Bmal1 in the circadian control of the HDM-mediated lung inflammation. Moreover, knockout of Bmal1 in macrophages alleviated the collagen deposition, mucus synthesis in bronchia, as well as the serum HDM-specific IgE production. These results suggest that myeloid Bmal1 plays an important role in the pathogenesis of chronic HDM-induced lung allergic responses. Further study will be focused on the mechanism and mediators from macrophages in the lung alveoli regulated by Bmal1 during the development of lung allergy.

NVMB4

Repurposing Thioridazine for Anti-atherosclerotic Treatment

JIANG Minchun, WANG Li, HUANG Yu

Supervisor: HUANG Yu

Atherosclerosis is a chronic arterial disease characterized by abnormal deposition of fatty and fibrous matter preferentially in the wall of curved and bifurcated arteries that are constantly exposed to low shear stress or disturbed blood flow. Our previous study demonstrated that endothelial YAP/TAZ activation induced by atheroprone-disturbed flow promotes inflammation and atherogenesis, whereas the athero-protective laminar flow inhibits YAP/TAZ by modulating the integrin–Gα13–RhoA pathway. Endothelial YAP/TAZ knockdown delays atherogenesis, indicating that YAP/TAZ holds promise as a novel drug target against atherosclerosis, as demonstrated by the YAP/TAZ-inhibitory effect of several anti-atherosclerotic and lipid-lowering drugs, especially statins. In the present study, we screen an FDA-approved drug library aiming to find clinical drugs that can target the YAP/TAZ signaling. Here, we show that thioridazine, an anti-psychotic drug, inhibits the activity of YAP/TAZ. Thioridazine downregulates the mRNA levels of YAP target genes (ANKRD1, CTGF, CYR61) in a concentration-dependent manner in human umbilical vein endothelial cells (HUVECs). Thioridazine also increases the phosphorylation of YAP and inhibits the nuclear translocation of YAP in HUVECs. Moreover, we observe that oral administration of thioridazine is able to reduce formation of atherosclerotic plaques in ApoE−/− mice on high fat diet. Taken together, the present results indicate that thioridazine might mitigate atherogenesis via inhibition of YAP/TAZ. Further experiments are being carried out to reveal detailed molecular mechanisms underlying the anti-atherosclerotic effect of thioridazine both in vivo and in vitro (this study is supported by RGC-CRF, No. C4024-16W and RGC-GRF No. 14164817).

NVMB5

PKD2-associated cardiomyopathies in 3D engineered cardiac tissue model.

Jingxuan LI, Xiaoqiang YAO

Supervisor: Xiaoqiang YAO

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong

Aims: Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in PKD1 and PKD2. Cardiovascular complication is the major cause of death in patients with ADPKD. However, it is still unclear of how PKD1 and PKD2 mutations could lead to cardiac disorders. Thus, in the present study, we aimed to elucidate the mechanism of PKD2 in cardiac disorders.

Methods: Human embryonic stem cells(hESC) were used to differentiate cardiomyocytes(hESC-CM). hESC-CMs were knockdown by shRNA-PKD2. 3D engineered cardiac tissues(3D-hvCTS) were used to assess PKD2-associated functional abnormalities in electrophysiological, Ca^{2+} handling and contractile properties.

Results: On day 7, functional analysis demonstrated reduced force production for *PKD2* knockdown hvCTS at all pacing frequencies, albeit neither of the observed differences were statistically significant when compared to scramble hvCTS. On day 11, *PKD2* knockdown hvCTS displayed a lower developed force during both spontaneous contraction and at all pacing frequencies. *PKD2* knockdown hvCTS presented with a slower maximum rate of force increase (+dF/dt) and maximum rate of force decrease (-dF/dt) compared to scramble controls during spontaneous contraction and at all pacing frequencies but no statistically significantly.

Conclusion: This study provides insight into the effects of *PKD2* knockdown on the contractility of hvCTS derived from hESC-CMs. The decrease in developed force produced by *PKD2* knockdown hvCTS suggests pathologies within hESC-CMs that impair contractility on a molecular scale.

NVMB6

Role of TRPC5 in endothelium-dependent contraction of hypertension mice model

Xiao Li, Chun-Yin Lo, Chi-Wai Lau, Xiaoqiang Yao

Supervisor: Xiaoqiang Yao

The augmented Ca^{2+}-related endothelium-dependent contraction (EDC) can stimulate the production of contractile prostanoids, which contributes to endothelial dysfunction of hypertension. However, very little is known about the molecular identities of endothelial Ca^{2+}entry channels that contribute to EDC. In the preliminary study, we found that endothelial cell transient receptor potential channel C5 (TRPC5), a Ca^{2+} -permeable channel, contributes to the EDC response by stimulating cyclooxygenase 2 （COX-2） activity in the carotid arteries of healthy mice. However, it is well known that EDC plays a more prominent role in some disease conditions such as hypertension. In this study, mouse model of hypertension was established by subcutaneous infusion of angiotensin II. Systolic and diastolic blood pressures were recorded by tail-cuff. Vascular tension was measured by wire myograph. EDCs were elicited by acetylcholine (ACH) in the presence of N^G-nitro-L-arginine methyl ester (L-NAME). Blood pressure was much larger in wild type (WT) mice than TRPC5 knockout (KO) mice. ACH-induced EDC in male mouse carotid arteries was also found to be substantially reduced in TRPC5 KO mice than in WT mice. ACH concentration from 0.3 to 3μmol·L^{-1} resulted in the contraction which could be abolished by a selective TP receptor antagonist S18886 at 100 nmol·L^{-1}. Based on the limited results, we demonstrated that an increased TRPC5 expression under hypertension will augment EDC response, which may further promote endothelial dysfunction.

NVMB7

PPARα activation inhibits endothelial cell inflammation and atherogenesis

Yujie Pu, Juan Huang, Chi Wai Lau, Chak Kwong Cheng, Jiang-Yun Luo, Yu Huang

Supervisor: Yu Huang

Background

Cardiovascular diseases (CVDs) remains a global health threat, especially in patients with diabetes or obesity. Although several therapies are currently available, the morbidity and mortality of CVDs are still high. Therefore, new therapies or the repurposing of existing therapeutic agents are urgently needed to better treat CVDs. Endothelial cells form the inner layer lining the lumen of all blood vessels and they play a crucial role in the maintenance of vascular homeostasis. Pro-inflammatory endothelial cell activation plays a pivotal role in initiating the formation of atherosclerotic plaques. Peroxisome proliferator-activated receptor alpha (PPARα) agonists are commonly used to treat patients with dyslipidemia and they may possess potential anti-inflammatory and anti-atherosclerotic effects. However, the mechanisms by which PPARα agonists work against atherogenesis remains unclear. This study aims to investigate the effect and mechanism of PPARα activation on endothelial cell inflammation and atherosclerosis.

Methods

To examine the effect of PPARα activation on the formation of atherosclerotic plaques, the PPARα agonist gemfibrozil was orally administered for 14 days to athero-prone *ApoE*$^{-/-}$ mice on a western diet. To investigate the beneficial effect of PPARα activation, both adenoviral and lentiviral vectors were used to control the PPARα expression in human umbilical vein endothelial cells (HUVECs) with and without treatment of proinflammatory cytokines, and then to assess the effect of agonists or antagonists on the PPARα activity and the expression of PPARα-regulated genes in HUVECs.

Results

The RNA sequencing analysis shows that in PPARα overexpressed HUVECs, most differentially expressed genes are found to be involved in metabolic and cardiovascular diseases while the enriched KEGG pathways are mostly related to the expression of pro-inflammatory genes. The results from *in vivo* animal study shows that 14-day oral administration of the PPARα agonist gemfibrozil inhibited the western diet-induced formation of atherosclerotic lesion and expression of several proinflammatory genes in *ApoE*$^{-/-}$ mice. These beneficial effects are partially reversed by co-treatment with PPARα antagonist GW6471, indicating that the anti-atherosclerotic effect of gemfibrozil is likely PPARα-dependent and mainly through suppressing vascular inflammation. Besides, genetic overexpression and pharmacological activation of PPARα protect HUVECs against IL-1β/TNFαinduced expression of pro-inflammatory molecules such as VCAM-1, E-selectin, and MCP-1, transcriptionally and translationally. On the contrary, PPARα knockdown by lentivirus-mediated shRNA or PPARα inhibition by GW6471 enhances IL-1β/TNFα-induced expression of proinflammatory genes in HUVECs.

Conclusion

In summary, the present study provides new evidence that PPARα activation is protective against the development of atherosclerotic lesions and this effect is most likely associated with downregulated expression of pro-inflammatory and atherogenic genes in endothelial cells. Further studies are underway aiming to provide more mechanistic insights into the potential of using PPARα agonists for the treatment of atherosclerotic vascular diseases [supported by HMRF (No. 07181286) and RGCCRF (C4024-16W)].

NVMB8

Exploiting the Random Forest classification algorithm for identifying features of muscle synergy post-stroke

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In stroke rehabilitation, there has been much interest in finding reliable neurological markers that can reflect motor impairment, predict functional gain from specific interventions, and shed light on basic mechanisms of post-stroke motor control. Various parameters dependent on muscle synergies – hypothesized modules of motor control – have been suggested as potential markers. Muscle synergies are often extracted from multi-muscle electromyographic signals (EMGs) using factorization algorithms, which decompose the EMGs into a set of time-invariant muscle synergies (*W*) and their temporal activation coefficients (*C*). Different analytic techniques have been employed to search for hidden *W*- and *C*-features that may be clinically useful. However, it remains unclear if any specific muscle-synergy features can serve as reliable predictive or diagnostic markers in diverse stroke survivors.

Our goal is to identify clinically and scientifically useful markers from features of EMGs and muscle synergies. We reason that any features that reliably distinguish the EMGs of a stroke-affected limb from those of a sound limb may be candidate markers. As a first step, we ask if a machine learning algorithm can discover muscle-synergy features that can be used for classifying whether a set of muscle synergies belongs to a stroke-affected or sound limb. To this end, we explore the utility of the Random Forest classification method, a supervised learning algorithm based on decision trees. In this method, an ensemble of decision trees is generated to avoid overfitting. Within each tree, features are randomly chosen, and a quantitative method is used to determine how well a feature can divide the test samples into different classes. Each tree then gives a vote to determine to which classes the data points belong, and the final classification is determined by combining the votes from all trees.

We tested the performance of the Random Forest in two datasets derived from chronic stroke survivors (N=15) and acute stroke survivors (N=28). Surface EMGs were collected from the paretic and sound sides (13 muscles each side) during 3D center-out reaching over 2-3 sessions, and EMGs from a total of 169 limbs were analyzed. Muscle synergies were extracted from the EMGs using non-negative matrix factorization. We found that the Random Forest could successfully classify the stroke-affected- and sound-synergy sets in the chronic dataset (~75% accuracy), but not the randomly shuffled synergy sets (~40% accuracy). This result is better than using the traditional K-means clustering method (~55% accuracy). The synergy features most frequently used by the

algorithm for classification include *W* components representing activations of biceps brachii (short and long heads), medial deltoid, trapezius (middle), and pronator teres. Our initial results here suggest that the Random Forest may be profitably employed for discovering new, potential markers during exploratory analysis of large datasets. The synergy features we identified also highlight how some muscle synergies may be altered in a predictable way at the chronic stage of stroke.

NVMB9

The Role of Endothelial PPARδ in Vascular Homeostasis

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Peripheral arterial disease (PAD) is a vascular disease occurs in the lower limb caused by ischemia due to atherosclerotic occlusion. It is a common vascular complication in patients with diabetes. Previous studies indicated potential function of PPARδ to enhance angiogenesis and inhibit inflammation, while the detailed mechanism is not fully understood. We aim to study whether endothelial PPARδ is important for vascular repair in mouse model of hindlimb ischemia and the possible mechanisms involved. Endothelial cell (EC) selective deletion of Ppard was achieved by Cre-loxP approach. Hindlimb ischemia was performed by ligation of the femoral artery, and blood flow recovery was monitored by Laser Doppler imaging. Low limb muscles from the injured and uninjured legs were used for flow cytometric, mRNA/protein expression, histology. Ppard deficiency in ECs from endothelial selective Ppard knockout ($Ppard^{EC-KO}$) mice attenuated the restoration of blood flow recovery over a two-week period in lean mice, which was further inhibited in obese mice fed with high fat diet. This effect is accompanied by reduced muscle mass, more severe inflammatory cell infiltration, fibrotic scar replacement of muscle fibers, less neovascularization and higher vascular leakage. Unresolved vascular inflammation in the ischemia muscle of $Ppard^{EC-KO}$ mice was accompanied by higher expressions of pro-inflammatory chemokines and adhesion molecules, and infiltration of macrophages and T lymphocytes. In mouse endothelial cell line, angiogenesis can be enhanced by PPARδ agonist and inhibited by Ppard siRNA. Our result indicate PPARδ is involved in vascular repair through multiple effects including enhancing angiogenesis and inhibiting vascular inflammation.

NVMB10

Mapping motor output units encoded in the human primary motor cortex with transcranial magnetic stimulation

XIE Jingping, Vincent C.K. CHEUNG

Supervisor: Vincent C.K. CHEUNG

Objective: The diversity of voluntary movements is achieved by the control of the central nervous system (CNS). Previous studies have suggested that the CNS generates movements by translating motor intentions into commands for a small number of motor output units, known as muscle synergies, representing co-activated muscle groups involved in different behaviors. However, physiological evidence of the muscle synergy in humans is not sufficient, and therefore the concept of muscle-synergy based movement generation has not been broadly accepted. Here, we aim to prove evidence to support the existence of muscle synergies in the human CNS by applying noninvasive electrical focal stimulation to the primary motor cortex (M1) thereby demonstrating the physiological significance of muscle synergies.

Method: Lower-limb and trunk muscles of one subject (16 muscles) were chosen to be target muscles, and the activities of these muscles were recorded by surface electromyography (EMGs). Simulation-evoked synergies were elicited by applying singlepulse transcranial magnetic stimulation (TMS) over the subject's scalp that corresponded to the M1 region. Besides, behavioral synergies were retrieved from different voluntary locomotor behaviors. The TMS-derived synergies were used to explain the behavior synergies. For each TMS-derived synergy, it's representation over M1 was also reconstructed by mapping its activities to the spatial locations of the stimulated M1 loci.

Result: We quantified the similarity between the TMS-derived and the behavioral synergies. The tms-derived synergies could be matched to behavioral synergies with high similarity.The topographic representation of the TMS-derived synergies also overlapped with each over over M1.

Conclusion: Our result demonstrates that it is possible to access and map out muscle synergies with the TMS technique, revealing how muscle synergies for locomotion are encoded in M1. Our data provide direct physiological evidence of the existence of muscle synergies in the CNS of humans. [III. Cardiovascular & Respiratory Systems][III-04. Cardiac: Cardiac hypertrophy & ischemia] a90631

NVMB11

Regulation of Orai1 in Angiotensin II-Induced Cardiac Hypertrophy

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Heart failure initiates with pathological cardiac hypertrophy which is a response of heart to increased workload. The cardiac hypertrophy is characterized as increased cardiomyocytes size, develops during the process of disorders such as hypertension and myocardial infarction. Calcium, as a second messenger, plays vital roles in mediating a wide range of cardiovascular diseases. Few studies reported store operated Ca2+ entry (SOCE) is associated with cardiac hypertrophy yet. Here, we hypothesized Orai1-mediated SOCE is responsible for the Angiotensin II-induced cardiac hypertrophy. Our data showed after the subcutaneous implantation of Ang II osmotic pump in C57BL6 mice, the heart size significantly increased. However, this effect could be abolished by knocking down Orai1 using AAV-Orai1-shRNA. A real-time PCR and western blots results showed the hypertrophic marker genes ANF, BNP, ß-MHC and cTnT upregulated in Ang II treatment group, while they remained nearly unchanged after the treatment of AAV-Orai1shRNA, indicating Orai1 could rescue the Ang II perfusion induced cardiac hypertrophy in vivo. Moreover, Masson's Trichrome staining convinced type I collagen levels in heart increased after Angiotensin II perfusion while it is attenuated after blocking Orai1, showing the progress of cardiac fibrosis during Orai1-mediated cardiac hypertrophy. Taken together, these findings suggest Orai1 as a novel regulator involved in Angiotensin II induced cardiac hypertrophy in vivo.

NVMB12

A Potential Crosstalk between YKL-40 and PTEN In Amyloid Beta Induced Signalling and Biological Effects in Alzheimer's Disease

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YKL-40 has been established to be a diagnosis biomarker of Alzheimer's disease (AD), and its increased immunoreactivity has been observed in astrocytes in the frontal cortex of AD patients. However, the functional role of YKL-40 in the process of AD is still not known. In our previous study, we have confirmed a cell-type specific relationship between YKL-40 and PTEN, an essential factor for long term depression and synaptic function. Here, we found significantly increased YKL-40 expression level in CSF of 5xFAD mice. By brain section confocal imaging, we found an astrocyte-specific expression pattern of YKL-40. Similarly, in primary cultured astrocytes, we observed increased YKL-40 expression in condition medium (CM) under amyloid beta (Ab) treatment. In contrast, knockout of PTEN reversed this Ab effect. Interestingly, by Western blotting analysis we found increased expression level of S6, p-70S6, p-AKT, mTOR, p-STAT3 but not PTEN in both 5xFAD mice and Ab treated wild type astrocytes. Since YKL-40 is more likely a secreted protein, we induced primary cultured neurons with YKL-40 and Ab. Immunofluorescent staining results showed that PTEN was recruited to synapses under Ab and YKL-40 treatment. In particular, by Western blotting analysis, we found increased expression of PTEN, YKL-40, synaptosin-1 and Caspase-3 following YKL-40 treatment, which confirmed the neurotoxic effects of YKL-40 on neurons. Also, this effect is most probably PTEN-dependent. In conclusion, our study for the first time, demonstrates that increased YKL-40 secretion activates AKT/mTOR, and JAK2/STAT3 pathway in a PTEN-dependent manner in astrocytes under Ab treatment, which may result in neuronal cell death.

Keywords: YKL-40, astrocytes, Ab, PTEN, Alzheimer's disease, neurotoxic
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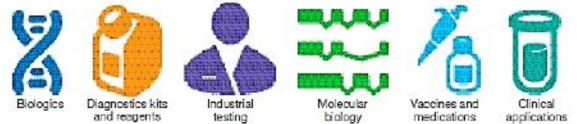

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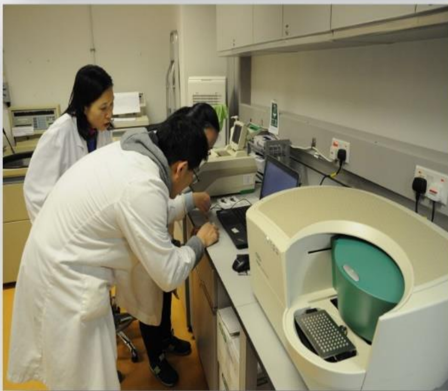
Glimpse of the SBS Core Laboratories

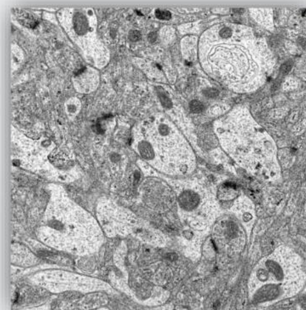
Core Lab Highlights: Induction Training for New Postgraduate Students, Equipment Training for Core Lab Users, New Technology Demo Workshop and Outreach Activities



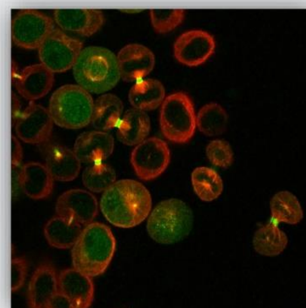
State-of-the-art Equipment and Some of Core Lab Work Results

The Ion Chef System & Ion Torrent S5 Sequencer

Seahorse XFe96 Extracellular Cell Analyzer



Centrifugal Concentrator



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