Category: Cardiovascular Disease

Department of Medicine

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<th>Principal Investigator</th>
<th>Project Title with a brief description</th>
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<tr>
<td>Prof Arthur Mark Richards</td>
<td>Dissecting cardiomyocyte cell cycle and proliferation in human healthy and diseased hearts, and mouse models of heart failure.</td>
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<td>Email</td>
<td><a href="mailto:mdcarthu@nus.edu.sg">mdcarthu@nus.edu.sg</a></td>
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Heart failure (HF) is a leading cause of mortality and morbidity, and a huge healthcare burden in modern society. Treatment for HF remains an inadequate stage-by-stage cumulative add-on therapy, and prognosis threatens to worsen as the world population is rapidly aging. Novel targets are urgently needed for the HF drug discovery pipeline. An underlying defect in HF patients is the progressive loss of cardiomyocytes (CMs), accompanied by functional derangements in contraction and relaxation. The regenerative capacity of the heart is limited by the inability of terminally differentiated CMs to adequately undergo cell division after the first weeks of life. However, recent studies have found that very low rate of cardiomyocyte turnover occurs in adult mouse and human hearts, mediated primarily by proliferation of pre-existing CMs. The evidence of human CM renewal suggests that the development of pharmacological strategies to stimulate this process may be a rational approach itself, or complement to cell transplantation strategies for CM replacement. Therefore, it is important to uncover gene regulatory pathways/drivers mediating the proliferation of pre-existing CMs in normal and failing human hearts. In order to tackle the important question, we will employ cutting edge technologies including single cell RNA-seq, live cell imaging, high-throughput content screening, CRISPR genome editing, and engineered heart tissue in both mouse and human models. Our project aims to: 1. profile genome-wide transcriptome of single cell healthy and diseased cardiomyocytes from mouse and human hearts, 2. discover unique and evolutionary conserved gene regulatory pathways specific for distinct subsets of cardiomyocytes relevant to cardiac regenerative potential, 3. functionally validate selected candidates to induce the proliferation of pre-existing cardiomyocytes. -----------------------------------------

Assoc Prof Lee Chi Hang, Ronald

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Mandibular Advancement Device for treatment of Obstructive Sleep Apnea and its Impact on Cardiac Remodeling (MOSAIC) - A Randomized, Double-blind, Placebo-controlled, Cross-over Trial

Primary aim: The MOSAIC trial aims to assess the impact of a
Telephone Number 91263120

Dissecting cardiomyocyte cell cycle and proliferation in human healthy and diseased hearts, and mouse models of heart failure

Heart failure (HF) is a leading cause of mortality and morbidity, and a huge healthcare burden in modern society. Treatment for HF remains an inadequate stage-by-stage cumulative add-on therapy, and prognosis threatens to worsen as the world population is rapidly aging. Novel targets are urgently needed for the HF drug discovery pipeline. An underlying defect in HF patients is the progressive loss of cardiomyocytes (CMs), accompanied by functional derangements in contraction and relaxation. The regenerative capacity of the heart is limited by the inability of terminally differentiated CMs to adequately undergo cell division after the first weeks of life. However, recent studies have found that very low rate of cardiomyocyte turnover occurs in adult mouse and human hearts, mediated primarily by proliferation of pre-existing CMs. The evidence of human CM renewal suggests that
the development of pharmacological strategies to stimulate this process may be a rational approach itself, or complement to cell transplantation strategies for CM replacement. Therefore, it is important to uncover gene regulatory pathways/drivers mediating the proliferation of pre-existing CMs in normal and failing human hearts. In order to tackle the important question, we will employ cutting edge technologies including single cell RNA-seq, live cell imaging, high-throughput content screening, CRISPR genome editing, and engineered heart tissue in both mouse and human models. Our project aims to: 1. profile genome-wide transcriptome of single cell healthy and diseased cardiomyocytes from mouse and human hearts, 2. discover unique and evolutionary conserved gene regulatory pathways specific for distinct subsets of cardiomyocytes relevant to cardiac regenerative potential, 3. functionally validate selected candidates to induce the proliferation of pre-existing cardiomyocytes.

Department of Surgery

Principal Investigator  Project Title with a brief description

Dr Wang Jiongwei  Targeting the interface between innate immunity and coagulation in cardiovascular disease with heart failure animal models

Email surwang@nus.edu.sg  Heart failure is a serious clinical syndrome caused by reduced pump function of the heart. Due to rapid ageing and increasing underlying causes like diabetes and hypertension, heart failure is the most rapidly increasing cardiovascular disease in loss of productivity and direct medical cost over the next 20 years. The most common cause of heart failure is acute myocardial infarction (MI). Coagulation (blood clot formation) and innate immune response (inflammation) are known to interact and worsen the disease progress to heart failure, however, effective treatment targeting the two remains limited. Therefore, further understanding of the pathological mechanisms and development of novel therapeutics are highly demanded for post MI treatment. In this project, we aim to: 1) determine the association between coagulation activity of patients’ plasma (and circulating exosomes) and disease progress in patients after MI; 2) explore the pathological mechanisms to look for novel therapeutic targets by using mouse heart failure models with genetic modified animals; 3) targeted delivery of potential therapeutics with various nano-drug delivery systems in mouse and possibly also in pig models (to get a rough idea, please refer to http://www.sciencedirect.com/science/article/pii/S0168365916307593) . The student is expected to work in a multidisciplinary and dynamic team, and to go abroad for a short training when necessary, to carry out
the study. Good communication skills and independence are required. The candidate will be trained for animal experiments, in vivo & in vitro imaging techniques (ultrasound, IVIS and confocal etc) and various molecular biology skills for this project.

Category: Cancers

Department of Anatomy

Principal Investigator
Assoc Prof Yip Wai Cheong, George

Project Title with a brief description
Evaluation of proteoglycans in breast cancer

Breast cancer is the commonest malignancy among women in Singapore and other parts of the world. Further, it is the most frequent cause of cancer deaths. Proteoglycans consist of one or more glycosaminoglycan chains attached to a core protein backbone. These molecules have been shown to regulate the activities of many different cancers. In this project, the expression and functional roles of various proteoglycans in breast cancer will be examined both in vitro and in vivo. In addition, the discoveries using these models will be verified by studies on breast cancer patient tissues.

Department of Biochemistry

Principal Investigator
Dr Chen Ee Sin

Project Title with a brief description
Epigenetic control of chromatin compaction to determine anti-cancer drug efficacy.

Epigenetic drugs have been increasingly used in the clinics for cancer treatments. This is especially the case a class of inhibitors that target the enzymes called histone deacetylases, which modulate the chromatin architecture that underlie epigenetic inheritance of developmentally regulated genes. In this project, we will investigate the interaction of histone deacetylase inhibition on gastric cancer cells via the modulation of DNA damage response and activation/deactivation of critical signal transduction pathways that control cell proliferation. This project also contains a section in which student will use the powerful genetic model fission yeast to interrogate detailed conserved molecular mechanisms of the histone dactylases in chromatin regulation.
Decipher the effect of different environment cues in regulating the tumour cell states equilibrium in breast carcinoma

A better understanding of the biology of metastasis is the key to improving survival in breast cancer patients. In this regard, insights into how different cancer cell states evolve and coexist within tumours could facilitate the development of more effective therapies. The breast carcinoma cell lines CA1a which is representative of Basal-like molecular subtype of breast cancer, and therefore present an excellent model to study this disease will be used. Preliminary data provided evidence that CA1a, contains CS-like cells, identified by the CD24low/neg EpCAMhigh/medium or low cell surface markers, as well as non-CS cells exhibiting CD24posEpCaMhigh phenotype. Interestingly, the equilibrium between the different cell states within the CA1a cell line could be shifted in response to external environmental cues from the culture medium. The present study will characterize phenotypically distinct subpopulations of cancer cells associated with Basal-like breast tumours, and decipher the effect of different environmental cues in regulating the cell states’ equilibrium.

Molecular and Translational WBP2 Oncogene Research

WBP2 was first found to be associated with breast cancer through phosphoproteomics analysis of a xenograft-derived isogenic breast cancer progression model(1). It was discovered in the same study to be hyper phosphorylated as breast cancer develops and demonstrated to be an authentic substrate of EGFR signaling. Subsequently, it was established in our lab to be a novel breast cancer oncogene that drives cancer in part by activating the ER and WNT signaling pathways (2). To understand how WBP2 is regulated, we performed protein interaction studies using mass spectrometry and identified ITCH E3 ligase as a novel binding partner of WBP2. We found that oncogenic WNT signaling promotes breast cancer by blocking the binding and destruction of WBP2 by ITCH (3). Recently, we performed RNA-seq and proteomics analyses and identified key pathways and proteins that are potential downstream effectors of WBP2 oncogenic actions. The objective of the project is to understand the modes of actions of WBP2 by studying how these downstream targets mediate the oncogenic effects of WBP2. The findings would provide opportunities for us to exploit scientific discoveries for cancer therapy. LYP lab has been successful in filing 30 patents, of which 10 have been awarded. Of the 10 - 4 have been licensed and 3 commercialized. References 1. Chen, Y., et.al & Lim, Y. P. (2007) Differential Expression of Novel Tyrosine Kinase Substrates during Breast Cancer Development. Mol Cell Proteomics 6, 2072-2087 2. Lim, S. K., et. al., and Lim, Y. P. (2011) Tyrosine phosphorylation of transcriptional coactivator WW

**Dr Long Yun Chau**

The role of amino acids in growth factor signaling

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FGF19 is a hormone-like FGF that is released by the small intestine in response to a rise in bile acid levels during the post-prandial state. FGF19 plays a critical role in cancer as well as metabolic signaling. Upon its release, FGF19 will travel to the liver via the hepatic portal vein. It is known that FGF19 activates hepatic anabolic metabolism (biosynthesis) which is required for cell growth, but the precise mechanism of this regulation remains poorly understood. Although previous studies have shown that FGF19 activates components of the protein translational machinery through the mitogen-activated protein kinase (MAPK) signaling pathway, the role of mechanistic target of rapamycin complex 1 (mTORC1) in FGF19 action remains unclear. This study will characterize whether FGF19 also mediates its effect through mTORC1 via activation of its downstream target P70S6K. This project will evaluate the contribution of canonical ERK/P90RSK or the PI3K/AKT pathways in the regulation of FGF19 action. Although growth factors are known to activate cell growth, this project will characterize the impact of amino acids on FGF19 action. We will address the fundamental question in biology – what kind of signaling will a growth factor induce in the absence or presence of nutrient availability? How does nutrient such as amino acid or glucose modulate the action of growth factor?

**Dr Tay Mei Sian, Yvonne**

Deconvoluting the role of the non-coding transcriptome in cancer

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Recent studies have revealed complex interactions among various RNA species, namely mRNAs and the noncoding transcriptome that includes snoRNAs, IncRNAs and pseudogenes. These RNA transcripts communicate and regulate each other by competing for binding to shared microRNAs on sequences known as microRNA response elements (MREs). As such, RNA transcripts function as competitively endogenous RNAs (ceRNAs) that form an intricate layer of post-transcriptional gene regulation. With computational predictive tools, transcriptome-wide biochemical screens and subsequent experimental validation, we aim to identify potential transcripts from the non-coding transcriptome that may similarly regulate critical tumor suppressors and oncogenes via ceRNA activity. By doing so, functional characterization of the noncoding transcriptome can be achieved. The understanding of this crosstalk can lead to new insights into the gene
regulatory properties of the non-coding transcriptome and identification of novel tumor suppressor genes or oncogenes which may represent attractive therapeutic targets in various human diseases.

**Department of Medicine**

**Principal Investigator**  
Dr Chester Lee Drum

**Project Title with a brief description**  
Mass spectroscopic biomarker discovery for individualized prediction of pharmacokinetics and disease.

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The way in which different ethnicities and individuals metabolize drugs can be represented as an intricate web of biochemical changes to prescribed medicines. When this variance in pharmacokinetic metabolism is cast against an electronic medical record database that documents both adverse drug reactions and beneficial effects, the total data set can be treated as a discovery mechanism for unexpected benefits and problems relevant to commonly prescribed medicines. In this project you will interface with a dedicated triple quadrupole tandem mass spectrometer and Q-ToF high resolution LCMS to develop novel detection methods for interesting drug metabolites and use this information to dissect exposure dependent effects of the measured drugs and their ability to effect markers of disease. Using established, large market medicines in new ways and understanding hidden risks and benefits to the Asian market will be critical to healthcare in the upcoming decades. This project seeks to position the graduate candidate to take advantage of this rapidly expanding scientific and regulatory field and develop skills relevant to the implementation of large-market pharmaceutical safety and effectiveness strategies. Large clinical datasets have already been recruited; because this project can be relevant to both cardiovascular and cancer therapies, the ultimate experimental design will tightly incorporate the intellectual goals of the student in addition to leveraging opportunities already established within the lab. You will work directly with a practicing physician (M.D., Ph.D.) and a team of mass spectroscopy and bioinformatics experts to complete both publications and your thesis.

**Department of Microbiology and Immunology**

**Principal Investigator**  
Assoc Prof Liu Haiyan

**Project Title with a brief description**  
The function and mechanism of IL-1a secretion in the tumor microenvironment
Interleukin-1α (IL-1α) is one of the most important inflammatory cytokines involved in inflammation and tumor development. Epithelial cell-expressed IL-1α plays a critical role during the initiation and maintenance of inflammation which is closely related to the development of many tumors. IL-1α presents as multiple forms in vivo, including precursor, propiece, membrane and secreted (mature) forms. We have recently demonstrated in a hepatocellular carcinoma (HCC) model that membrane IL-1α inhibits tumor development via promoting T and NK cell activation (Cancer Research 2016 76(11):3179). However, it has been shown that IL-1α released from the necrotic hepatocytes could contribute to liver carcinogenesis by stimulating compensatory proliferation of the hepatocytes. Our preliminary results also suggest that secreted IL-1α in the tumor microenvironment could facilitate tumor growth and suppress anti-tumor immune response by recruiting MDSCs. Therefore, regulating IL-1α release is critical for modulating inflammatory tumor microenvironment and could be a target for tumor immunotherapy. However, the immune regulatory function of secreted IL-1α and the mechanism modulating IL-1α secretion during tumor development remain unknown. We hypothesize that precursor IL-1α could be actively cleaved by calpains during inflammatory conditions and tumor development, which facilitates the release of IL-1α from the cells. The secreted IL-1α could recruit MDSCs through the induction of other cytokines and chemokines to suppress anti-tumor immune response and promote tumor progression. In the proposed study, we will: (1) Determine the immune regulatory role of secreted IL-1α during tumor development; (2) Identify the calpains that cleave IL-1α precursor to facilitate IL-1α secretion during tumor development; (3) Investigate the regulatory mechanisms of IL-1α release during tumor development. Understanding the immune-regulatory mechanisms of secreted IL-1α and how the release of IL-1α is controlled are critical in designing immunotherapeutic strategies targeting the different patterns of IL-1α expression to modify the inflammatory tumor microenvironment.

Design, generation, and investigation of novel dumbbell-shaped DNA minimal vectors for trans-splicing based suicide gene therapy targeting virus infection or cancer

The concept of suicide gene therapy is based on the delivery of a genetic vector that leads to selective destruction of aberrant target cells. We developed a RNA trans-splicing-based Herpes simplex virus thymidine kinase (HSVtk)/ganciclovir (GCV) suicide gene therapy approach that can be programmed to target any disease biomarker. This approach was successfully tested in tissue culture cells were we achieved selective destruction of liver cancer cells or cells irreversibly transduced with the human papillomavirus type 16 or with HIV-1. The
The aims of this project are firstly to further optimize the suicide gene therapy sequences with regard to on-target activity and specificity. Secondly, novel dumbbell-shaped DNA minimal vectors will be designed and generated to deliver the therapeutic sequences into target cells. Dumbbell vectors are safe non-viral vectors which were proven to trigger profound transient gene expression even in human primary cells. We will investigate the intracellular fate of these vectors including potential genomic integration, chromatinization, and immune sensing. Finally, dumbbell-shaped suicide vectors together with a suitable transfection reagent will be tested in more clinically relevant settings such as in three-dimensional spheres formed by tissue culture cells or human primary cells using a microfluidic device or in animal (mice) models. This project is highly translational offering promising perspectives towards suicide gene therapy of cancer or virus infection.

**Department of Obstetrics & Gynaecology**

**Principal Investigator**  
Assoc Prof Fong Chui Yee

**Project Title with a brief description**  
The therapeutic potential of stem cells from the Wharton's jelly of the human umbilical cord

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Wharton's jelly of the human umbilical cords are a novel source of stem cells for tomorrow's cellular therapy. Wharton's jelly stem cells (hWJSCs) are non-controversial and can be harvested in abundance from discarded human umbilical cords. They have numerous advantages and have many potential therapeutic uses such as (1) they have tumericidah properties, (2) they can be differentiated into many types of tissue for tissue regeneration and (3) they can ex vivo expand haematopoietic stem cells. This project will target on one specific therapeutic use and study in depth into the mechanisms and translational application to benefit patients.

**Department of Pharmacology**

**Principal Investigator**  
Assoc Prof Gautam Sethi

**Project Title with a brief description**  
Identification of a novel agent that can suppress proliferation, induce apoptosis and overcome chemoresistance in hepatocellular carcinoma

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in association with the dissemination of hepatitis B and C virus infection, hepatocellular carcinoma (HCC) is the fifth most common cancer and causes more than 600,000 deaths annually worldwide. Although surgery remains to be the first choice for HCC, tumor size,
hepatic functional reserve and/or portal hypertension may all restrict surgical ablation. Currently, first line drugs used for HCC include fluorouracil, cisplatin, doxorubicin, paclitaxel and mitomycin, but most of these are non-selective cytotoxic molecules with significant side effects. Therefore, the need to develop novel therapeutic strategies for HCC is of paramount importance. STAT3, a member of the signal transduction and activation of transcription (STAT) family, is constitutively active and regulates the expression of number of genes that play a pivotal role in the proliferation, survival, metastasis and angiogenesis in HCC. Hence, STAT3 is considered as an attractive therapeutic target for the treatment of HCC patients. Thus the project aims to identify a novel agent that can inhibit proliferation, induce apoptosis and overcome chemoresistance in different HCC cell lines. The agent will be identified by screening small molecule inhibitors library available in the laboratory. The effect of identified agent on signaling pathways that contribute to survival and chemoresistance in HCC will also be investigated in detail. Furthermore, detailed investigation, using, in vivo HCC mouse models will be carried out.

**Targeting oncogenic transcription factors to overcome chemoresistance in breast cancer**

More than 900,000 women are diagnosed with breast cancer worldwide every year. Despite advances in earlier detection and therapy for breast cancer, it is the development of metastatic disease that is the principal cause of death, with bone, lung, lymph node, and brain being the most common sites of metastasis. The predominantly lytic nature of breast cancer bone metastases is thought to be a consequence of a vicious cycle, in which metastatic cells release growth factors and cytokines that promote osteoclast activation and bone destruction; this in turn liberates growth factors sequestered in the bone matrix, which then promote growth of the cancer cells. Hence, new agents that are safe and effective are urgently needed. The use of anti-cancer drugs derived from natural sources may be able to overcome resistance without some of the debilitating side effects of conventional chemotherapy. Between 1981 and 2002, 48 of 65 of all drugs approved for the therapy of cancer were natural products, were based on natural products, or mimicked natural products in one form or another. How they fight disease and their molecular targets are under continual investigation. Thus the project aims to identify a novel agent that can inhibit proliferation, induce apoptosis and overcome chemoresistance in breast cancer cell lines. The agent will be identified by screening natural products library available in our laboratory. The effect of identified agent on signaling pathways that contribute to survival and chemoresistance in breast cancer will also be investigated in detail using in vivo mice models. Overall this project will lead to discovery of a novel drug that can help
to enhance treatment efficacy, reduce toxicity, and overcome chemoresistance in breast cancer.

### Department of Physiology

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<th>Project Title with a brief description</th>
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<td>Assoc Prof Lim Hsiu Kim, Lina</td>
<td>Stress and its role in cancer and the tumor microenvironment</td>
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<td>High stress levels have long been known increase risk of cardiovascular disease and stroke, and cause premature aging. However, studies relating to stress and cancer development are less common, and mostly focus on the cancer cells themselves. As my lab has been focusing on the tumor microenvironment and the importance of macrophages in the promotion of tumor growth, it is of interest to us to investigate the effect of stress and stress hormones in the tumor microenvironment. We have made significant progress to understand how macrophages in the tumor microenvironment enhance cancer growth and how tumors induce macrophage polarization to aid in its growth. We have shown that breast cancer cells secrete chemokines and mediators to promote macrophage polarization to an M2 phenotype. This is dependent on anti-inflammatory annexin-A1 (ANXA1), which is highly expressed in metastatic and triple negative breast cancers. We hypothesize that stress (acute or chronic) induces the production of stress hormones which can modulate the tumor microenvironment consisting of tumor cells and macrophages to promote tumor growth and metastasis through ANXA1 and downstream signaling. Our preliminary data show that ANXA1 deficient mice grow slower tumors which do not metastasize, and old ANXA1 deficient mice are less anxious and more exploratory than normal mice. Furthermore, we show that social stress in mice indeed enhances tumor growth. In this project, we will focus on how different levels and types of stress and stress hormones influences immune cells and tumor cells to enhance tumorigenesis and if inhibiting stress receptors will be a novel therapeutic avenue in the treatment of cancer in the future.</td>
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<th>Dr Ong Sek Tong, Derrick</th>
<th>To identify epigenetic regulators that influence glioma stem cell (GSC) self-renewal and tumorigenicity.</th>
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<td>Aberrant brain stem cell behaviour not only affects aging but also cancer. In gliomagenesis, a sub-population of neural stem/progenitor cell (NSPC)-like glioma cells (so-called ‘glioma stem cells’ or GSC), shows uncontrolled self-renewal activity, robust tumorigenic potential and therapeutic resistance, contributing to the pathogenesis of</td>
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Glioblastoma Multiforme (GBM). Although aberrant epigenetic changes, including mutations of epigenetic regulators, global DNA hypomethylation and alterations in histone modifications, have been described in GBM and there is evidence that specific chromatin states impact on GSC cell identity and tumorigenicity, the epigenetic basis for GSC self-renewal and tumorigenicity remains ill-defined. To that end, we will perform an unbiased in vivo ORF screen to discover epigenetic regulators that promote tumorigenicity of pre-malignant human NSPC, followed by the identification of epigenetic regulators that influence GSC self-renewal potential using the in vitro limiting dilution tumorsphere and in vivo limiting dilution tumor initiation assays. The insights gained have direct implications in the development of actionable therapeutics to treat GBM. Students can look forward to learn intracranial injection procedure, GSC assays, various –omics (including RNA-Seq, ChIP-Seq and RRBS), flow cytometry and histological analyses.

### Dr Tee Wee Wei

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**Targeting epigenetic and transcriptional dysfunction in cancers**

A major effort in the lab is to investigate how transcriptional and chromatin aberrancies promote tumorigenesis and to identify new epigenetic targets in cancer. Notably unlike genetic mutations, epigenetic alterations are reversible. This presents a therapeutic opportunity to restore proper gene expression patterns and revert diseased phenotypes towards normality. We have performed integrative analyses of cancer epigenomic data with genome and transcriptome sequencing data, and identified several potential chromatin regulators that are implicated in tumorigenesis. The student will carry out an in-depth study of the mechanisms by which these chromatin regulators promote tumorigenesis as well as evaluate the druggability potential of these factors. He/She will be exposed to various cancer-specific assays, as well as 'omics' technologies, both computational and experimental. For more information, please see: https://wwteelab.com or http://www.imcb.a-star.edu.sg/php/wwt.php

**Category:** Metabolic Diseases Research

**Department of Obstetrics & Gynaecology**

**Principal Investigator**
Assoc Prof Chan Shiao-Yng

**Project Title with a brief description**
Placental lipid flux in gestational diabetes as a potential therapeutic target in mitigating the transgenerational cycle of...
Gestational Diabetes mellitus (GDM) is glucose intolerance that is first recognized during pregnancy and is linked with adverse obstetric outcomes and future offspring risk of obesity. In Singapore, GDM complicates 20-25% of pregnancies and diabetes accounts for over 10% of disease burden in the general population. The placenta is central in determining the in utero environment within which the fetus develops. It ensures that fetal requirements are balanced with maternal resources available to support the pregnancy using a range of signaling pathways. The placenta plays a large part in transmitting cues to the developing fetus to prepare for the challenges of the environment it could face following birth by imposing a "programming" effect that is sustained into ex utero life. Such evolutionary mechanisms could have unintended consequences in pathological conditions like GDM. We hypothesise that a critical biological pathway through which this occurs is the regulation of placental lipid flux. Lipids are not just structural components of cell membranes but also biological signals in their own right. Evidence for a significant alteration in the uptake, metabolism and release of lipids during the maternal-fetal lipid transfer across the placenta is starting to emerge in GDM and could be a potential intervention target. In this study, we aim to use a range of ex vivo and in vitro techniques to better understand the specific dysregulation occurring in lipid flux within placenta from GDM pregnancies and investigate how this could be manipulated by various nutritional supplements and drugs. Results will also be correlated with a range of clinical characteristics measured longitudinally during pregnancy and later in the offspring, and will provide an opportunity to investigate and understand the placental mechanisms underlying altered offspring growth and development. The evidence gained from this study could potentially lead to effective prevention and intervention strategies for GDM.

Assoc Prof Fong Chui Yee

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Studies on the effects of gestational diabetes mellitus on the fetus using stem cell derived tissues

Gestational Diabetes mellitus affects 20-25% of pregnancy. Infants of such diabetic mothers have an increased risk of obesity, metabolic syndrome, hypertension and cardiovascular disease. Epidemiology studies show that the consequences of prolonged hyperglycemic exposure are life-long and even extend to the next generation through the maternal line. Using stem cell technology, we wish to generate tissues that would mimic those of the infants to study the effects of maternal hyperglycemia. Novelty of this project would be that an in vitro model can be developed using stem cell technology to (1) study the health status of the infant and (2) prepare immunologically
compatible tissue of the infant for cell-based therapy.

Category: Infectious Disease

Department of Medicine

Principal Investigator  Project Title with a brief description

Dr Catherine Ong Wei Min  Diabetes mellitus and the regulation of AMPK in the immunopathology of Tuberculosis

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Tuberculosis (TB) infects a third of the world's population while incidences of diabetes is increasing. Patients with diabetes and TB are more likely to develop cavities, and are more likely to have a higher bacterial burden. AMP-activated protein kinase (AMPK) is a master regulator of cellular metabolism including glucose metabolism and has been found to regulate proteases driving tissue destruction in TB. The hypothesis of this project is that hyperglycaemia increases tissue destruction in TB. The specific aims are to: 1) Investigate the role of hyperglycaemia in the replication of M.tuberculosis/BCG and in infected primary human innate cells 2) Investigate hyperglycaemia in the regulation of proteases in TB 3) Investigate the role of AMPK in patients with and without diabetes in a cellular model of TB First, the effect of glucose would be examined for a dose-dependent response on BCG/M.tuberculosis (M.tb) growth by colony forming units and by spectrophotometer. Primary human monocytes and neutrophils would be extracted using Pluriselect, a bead-based technique, and infected with live BCG or live virulent H37Rv. Intracellular survival of mycobacterium in increasing concentrations of glucose will be evaluated, as will reactive oxygen species from neutrophils using FACs or quantitative fluorescent assay, neutrophil extracellular trap formation by Picogreen, and proteases such as matrix metalloproteinases by luminex assay. Matrix degradation will be evaluated using DQ matrix assays by fluorometry. Finally, the data will be validated in patients with and without diabetes. Effects of AMPK activators metformin and AICAR and Dorsomorphin an AMPK inhibitor will be evaluated on the secretion of proteases. This project will provide a broad-based training in crucial immunology techniques, and will dissect the mechanisms of increased immunopathology in patients with diabetes and TB at the translational interface. It would provide preliminary data and will serve as an excellent platform to proceed onto a PhD.
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<tr>
<th>Dr Swaine Lin Chen</th>
<th>Email</th>
<th><a href="mailto:mdcslc@nus.edu.sg">mdcslc@nus.edu.sg</a></th>
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Enabling sexual genetics in bacteria to study the evolution of virulence and antibiotic resistance

A common question that we often cannot answer is why certain people get sick and others don't when exposed to the same bacteria or virus. On the other side of the coin, sometimes closely related bacteria differ in their ability to cause disease in the same patient. We don't have good ways to answer the second question for many bacteria. This proposal uses recently developed genetic tools in my laboratory combined with advances in analysis of DNA sequences to finally have a way to answer why closely related bacteria, particularly Escherichia coli (some of which can cause diarrhea or urinary tract infections and others of which are normal residents of the guts of nearly all humans), differ in their ability to cause disease. This method is very general and can also be applied to understanding why some bacteria are more resistant to antibiotics than others; this is important because antibiotic resistance is increasing and, if unchecked, may bring us to a time when simple infections and procedures result in lethal and untreatable infections.

Department of Microbiology and Immunology

Principal Investigator | Project Title with a brief description
--- | ---
Assoc Prof Chow Tak Kwong, Vincent | Activation and formation of neutrophil extracellular traps in severe influenza and secondary pneumococcal pneumonia

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--- | ---
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Patients infected with highly pathogenic influenza viruses (e.g. H5N1) develop signs of acute lung injury (ALI) with pulmonary infiltration and edema, which further progress into acute respiratory distress syndrome (ARDS) and respiratory failure. Neutrophils play key roles in acute lung injury by producing reactive oxygen intermediates, inflammatory cytokines and enzymes such as elastase, or via formation of neutrophil extracellular traps (NETs), which can cause alveolar destruction and pulmonary edema. Although highly pathogenic viral and bacterial infections cause extensive accumulation of neutrophils in the lungs, how neutrophils mediate the progression of clinical manifestations leading to acute lung injury is not well-understood. This project will explore the molecular mechanisms by which neutrophils contribute to ALI and ARDS in mouse models infected with influenza A H1N1 and H3N2 viruses, as well as in secondary pneumococcal pneumonia. The objectives include: (1) Evaluate progressive changes in the lungs and contribution of neutrophils and their mediators in pulmonary complications of ALI/ARDS during primary and secondary pneumonia. (2) Employ live imaging techniques to investigate the major sources, migration and activation of neutrophils into the lungs,
and whether targeting key cytokines/chemokines can ameliorate NETs
development and pathogenesis. (3) Explore opportunities for
intervention by treatment with inhibitors against pneumococcal
virulence factors and vascular leakage which will be tested in
combination with antimicrobial agents – this will evaluate whether
such interventions alleviate complications of pulmonary edema and
alveolar injury. (4) Compare and characterize pulmonary NETs
formation and transcriptomes in response to infection with different
strains of influenza virus and Streptococcus pneumoniae of varying
virulence, and whether antimicrobial combination therapy can
ameliorate their deleterious effects. (5) Identify host signature genes
that can predict progression from pneumonia to sepsis. References:

Generating antiviral neutralizing antibodies and characterizing
their mechanism of inhibition

Seasonal influenza A virus causes significant morbidity and mortality
yearly while newly emerged strains continue to pose pandemic threats.
Current strategies against influenza include vaccination and antiviral
drug treatment. However, predicting the major strain that may cause
the next pandemic is the main obstacle in current vaccine development.
Moreover, some viruses have acquired resistance to approved antiviral
drugs. Passive immunotherapy is now increasingly being used to treat
human infectious diseases and there is a demand for the development
of neutralizing mAbs for passive immunotherapy in the event of a
highly pathogenic flu pandemic as this could be particularly useful for
protecting certain groups of people, such as immuno-compromised
patients or the elderly, who may not respond well to vaccines. As the
hemagglutinin (HA) protein mediates viral entry, it has been the main
target for the preclinical studies on antibody-based immunotherapy and
these studies suggest that it may be a viable option to administer
neutralizing HA mAbs as a form of passive immunotherapy for
influenza A infection. However, viral escape mutants were observed
when anti-HA mAbs were used individually. Combination therapy,
where multiple steps in the virus life cycle are inhibited
simultaneously, is highly recommended to minimize the development
of escape viruses. Hence, this study uses a multi-disciplinary approach
to determine if other viral proteins of influenza A virus can stimulate
neutralizing antibodies that prevent viral infection and/or replication. If
we can develop combination therapy by using a mixture of mAbs that
bind to different components of the virus, this will minimize the chance
of drug resistant virus developing and this will help in the
establishment of a novel class of antiviral therapeutic drugs.

**Virus-host interaction in hepatitis B virus replication and pathogenesis**

Approximately 2 billion people have been infected hepatitis B virus (HBV) which primarily attack the liver. While some of the infected people clear the virus, a high percentage of them becomes chronically infected. Subsequently, the majority of them develop severe liver disease which includes fibrosis, cirrhosis and eventually hepatocellular carcinoma (HCC). While it is known that infection by HBV contributes directly and indirectly to the development of HCC in patients, the exact manner by which each virus participates in this complex process is not completely understood. During this process, the complex interplay between viral proteins and host cell machineries contributes to viral replication and/or pathogenesis. We will be using various approaches to identify novel viral-host interactions and determine how each host factor interacts with viral protein(s) and at which step(s) of the viral life-cycle. Detailed analysis will be performed by using infectious system and infectious viral clones will be used to make modified viruses in order to identify specific viral motifs in HBV genome that are required for effective replication. Identification and characterization of such crucial viral-host interaction therefore offers opportunities for designing new treatments for HBV.

**Department of Physiology**

**Principal Investigator**  
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**Project Title with a brief description**

**The cross-talk between Annexin-a1 and ERK/MAPK signaling pathway in influenza virus infection**

The goal of this study is to reveal the hitherto unexplored relationships between the protooncogenic EGFR/Ras/Raf/MEK/ERK pathway (hereafter the EGFR/ERK pathway), the Annexin-A1 protein and its receptor FPR2, and the etiology of the influenza disease. Exposing the relationships between these components may lead to a totally novel view of influenza’s etiology and therapy. Annexin-A1 and the EGFR/Ras/Raf/MEK/ERK cascade are critical components, involved in essentially every physiological process. Components of the cascade are mutated in nearly all cancer cases. It is intriguing therefore that their involvement in the etiology of influenza has not been addressed so far. Initial observations suggest that those molecules may be involved in influenza infectivity, calling for a systematic and comprehensive study. To assess the involvement of the
EGFR/Ras/Raf/MEK/ERK cascade and Annexin-A1 systematically we will establish an array of isogenic cell lines where using CRISPR/Cas9 techniques, Annexin-A1, FPR1, FPR2, H-ras, K-ras, B-Raf, MEK1, MEK2, ERK1, or ERK2 will be silenced. In another series we will express constitutively active variants. As all cell lines are derived from the same parental line, any difference between them could be legitimately attributed to the missing protein, or to the overactive one. All cell lines will be infected with influenza or synthetic analogues of RNA. Viral entry and propagation will be monitored immunologically, microscopically, by PCR and by testing the levels of various cytokines. Components found to affect influenza infectivity and propagation, will serve as targets for anti-influenza therapy.

Category: Neuroscience

Department of Anatomy

Principal Investigator: Assoc Prof Tay Sam Wah, Samuel

Project Title with a brief description: Implications of telomerase shortening in the recapitulation of age-onset neurodegenerative diseases

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Induced pluripotent stem cells (iPSCs) present as a powerful tool to simulate disease modelling and investigate the pathogenesis of neurodegenerative disorders where suitable animal models are lacking. However, their ability to recapitulate age-related disease onset still poses as a challenging feat. Often, differentiation protocols produce fetal-like state of cells rather than the fully mature state, in which most adult diseases inflict. The shortening of telomeres has been typically established as an indication of cellular aging. Telomere shortening can lead to the vulnerability of DNA damage; and even senescence and apoptosis of the cells. We would thus like to investigate the acceleration of age-onset neurodegenerative disease phenotypes like frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). We will also examine the implications of the induced aging process by telomere shortening during the differentiation of the iPSCs into motor neurons for ALS; and cortical neurons for FTD, and comparing the results obtained in vitro with that of the in vivo animal models. Therefore, in this project we propose to knock-out the telomerase reverse transcriptase (TERT) gene in wild type human iPSCs and isogenic ALS and FTD iPSC cell lines using the CRISPR/Cas9 technology and differentiate them along the motor neuron and the cortical neuron lineages, respectively. In doing so, we seek to uncover the differences in motor neuron health and observe the
different transcriptome dynamics by doing RNA-sequencing in “aged” cells. Subsequently, we would like to define the conservation of affected genes, transcripts, and pathways of our in vitro disease modelling compared with that of the in vivo mouse models. From this project, we expect to recapitulate age-related disease onset of these 2 neurodegenerative diseases and elucidate the mechanisms involved. The findings from this research project will have important implications for in vitro disease modelling of age-related neurodegenerative diseases in the future.

**Department of Pharmacology**

**Principal Investigator**

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**Project Title with a brief description**

**DR-region of Na+/K+ ATPase is a new target to treat stroke**

Stroke is a major cause of disability and death worldwide. Na+/K+ ATPase (NKA) activity is decreased in the brain of stroke patients. We developed an antibody against the DR region of NKA (DR-Ab), which not only stimulated NKA activity but also produced neuroprotective effects against neuronal cell death in ischemia. In the transient middle cerebral artery occlusion (tMCAO) mice, both pre- and post-stroke treatment with DR-Ab significantly decreased the infarction volume. We therefore hypothesize that DR-region of NKA is a new target to treat ischemic brain diseases and activation of NKA with DR-Ab may be useful to treat stroke. This project is designed to study the underlying mechanisms of the therapeutic effect of DR-Ab. Membrane NKA loss and excitotoxicity will be studied.

**Target Na+/K+ ATPase to treat Parkinson's Disease**

Parkinson's Disease (PD) is a chronic and progressive movement disorder. It is the 2nd most common neurodegenerative disorder after Alzheimer's disease. Na+/K+ ATPase (NKA) activity is decreased in neurodegenerative diseases. We developed and antibody against the DR-region of NKA (DR-Ab), which produced neuroprotective effects against 6-OHDA-induced dopaminergic neuron injury and significant therapeutic effects. This project is designed to study the underlying mechanisms including anti-oxidant, anti-inflammatory and anti-apoptotic effects. The interaction between NKA and alpha-synuclein will also be studied.

**Department of Physiology**
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<th>Principal Investigator</th>
<th>Project Title with a brief description</th>
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<td>Dr John Chua Jia En</td>
<td>Intracellular trafficking of proteins to the presynapse</td>
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Mammalian synapses are contact sites between neurons essential for cell-cell communication. Each synapse comprises over a thousand proteins that must be synthesised in the cell body and subsequently delivered to the synapse via the intracellular trafficking machinery. However, the mechanisms and regulatory pathways involved in synaptic protein transport remains largely unknown. Defects in intracellular transport have been observed in several important neurodegenerative disorders such as Alzheimer’s, Huntington’s, Parkinson’s and ALS. This highlights defective transport to synapses as a common impairment in these clinically important disorders. In this project, we will examine how different classes of presynaptic proteins are transported to synapses at different stages of neuronal development using live fluorescence microscopy techniques. We will label trafficking vesicles by generating and expressing differently colored fluorescently tagged markers corresponding to the various classes of presynaptic proteins in living neurons. Techniques involve include molecular, biochemical and microscopic techniques such as plasmid manipulation, primary neuronal cell culture and transfection, and immunofluorescence microscopy. Results from this study will contribute to our understanding of how intracellular transport supports synaptic functions in normal neurons and how its defect can ultimately lead to the onset of synapse dysfunction and degeneration.

| Assoc Prof Khanna Sanjay | An investigation into the medial septal basis of neuropathic pain and co-morbidity |

Chronic pain, especially neuropathic pain, has a very negative effect on lifestyle and can lead to situation where subjects are depressed and show an impairment of learning and memory. However, the existing drugs provide partial relief from pain, at best. The current investigation attempt to advance the understanding of the nervous system basis of chronic pain and identify the brain neurochemistry that underpins the negative effect of pain. The proposal will test the novel hypothesis that neurotrophic mediated neuroplasticity in the medial septum drives experimental neuropathic pain and co-morbidity. The medial septum is a forebrain structures which we have identified as crucial for the mediation of persistent pain. It is at the crossroad between the cortex and the midbrain. In the present investigation we will use behavioral, molecular biology and cellular/neural techniques to delineate the neurochemical/signalling changes in the medial septum and identify the neurons that are involved. The successful conclusion of the study will point towards fresh directions for translational investigations in

Dr Ling Shuo-Chien

Harnessing Anti-Aging Potential to Understand and Combat Adult-Onset Neurodegenerative Diseases

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Adult-onset neurodegenerative diseases remain the most devastating and crippling diseases without a cure. Our group investigate the mechanisms underlying amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), which affect the motor and cognitive systems, respectively. ALS and FTD are now recognized as representing two ends of the same disease spectrum with multiple common genetic loci implicated in ALS and FTD, including mutations in the TARDBP (encoding TDP-43), FUS/TLS (fused in sarcoma/translocated in liposarcoma), and C9ORF72 (chromosome 9 open reading frame 72) genes. Intriguingly, pathological aggregates of TDP-43 and FUS/TLS are the defining features of ALS and FTD, establishing a gene-pathology relationship that are commonly seen in all major neurodegenerative diseases. The research in my laboratory uses disease-causing genes in human to understand the normal and pathophysiological process by constructing in vitro and in vivo models followed by an integrated approach combining genomic quantitative analysis, molecular and cellular studies. We have generated and characterized several mouse models, including transgenic and conditional alleles for TDP-43, FUS/TLS and C9ORF72. This arm of our research is to understand the pathogenic mechanisms and identify therapeutic targets for ALS and FTD. As aging is the leading risk factor for age-associated diseases, including late-onset neurodegenerative diseases. Exciting discoveries reveal that aging is subjected to regulation by signaling pathways and specific factors, and slowing aging process postpone age-associated diseases. The second arm of our recent effort is to identify critical molecules involved in prolonging health- and life-span using the genomic dataset generated in the above-described approaches. Specifically, we identified altered expression of an anti-aging gene and signaling pathways. We are currently working to determine how these anti-aging pathways may regulate cognitive function, and to harness their potential to extend health- and life-span with a more youthful and disease-free aging process.
Mitochondria play an important role in intracellular Ca2+ homeostasis. Ca2+ influx across the IMM plays a fundamental role in the regulation of wide range of physiological processes, including shaping the amplitude and spatiotemporal patterns of cytosolic Ca2+ and cellular bioenergetics. Although basic mechanisms of mitochondrial Ca2+ homeostasis have been established, the molecular identities of mitochondrial Ca2+ uptake remained mysterious until very recently. We discovered MICU1 as a molecular component of uniporter that contributes to physiological matrix Ca2+ maintenance. MICU2, a paralog of MICU1 was shown to have a redundant role as MICU1. Loss of function mutations in MICU1 has been shown to have detrimental effect leading to brain and muscle disorder in humans. We have recently shown EMRE as a matrix calcium sensor that governs the gatekeeping of the MCU. Thus, the matrix calcium levels are kept in check by both MICU1/MICU2 complex in the intermembranous space and EMRE from the matrix side. Nevertheless, the role of mitochondrial Ca2+ homeostasis under resting conditions in excitable cells such as neurons remains largely unexplored. Further, the link between mitochondrial Ca2+ overload and mitochondrial ROS (mROS) generation in neurons remain largely unexplored. Thus, there is an urgent need for a better understanding of the basic mechanisms that regulate mitochondrial Ca2+ handling and mROS production that lead to neuronal dysfunction. Our preliminary investigation on the [Ca2+]m uptake machinery serendipitously identified a new role of MICU1 in protecting the MCU mediated [Ca2+]m overload and the resultant mROS overproduction. Interestingly, MICU3, a paralog of MICU1, is expressed exclusively in brain mitochondria and its functional role in mitochondrial Ca2+ signaling has never been studied. MICU3 possess highly conserved Ca2+ binding EF-hand domains similar to MICU1. The overarching goal of this proposal is to understand the basic molecular mechanism of MICU3 in neuronal mitochondrial Ca2+ homeostasis.

Category: Functional Ageing

Department of Orthopaedic Surgery

Principal Investigator

Prof Hui Hoi Po

Project Title with a brief description

Repair of Osteochondral Defects using a Combination Stem Cell and Heparan Sulfate Device
Damage to knee cartilage exposes underlying bone tissue, so causing significant pain and inflammation, often leading to osteoarthritis, which is one of five main causes of disability in Singapore. Worldwide, osteoarthritis incurs an estimated global expenditure of USD$18.5 billion annually, a figure growing rapidly. Current standard-of-care focuses on surgically drilling into the exposed bone tissue to create micro-fractures, which in turn release cells and growth factors to promote healing of the cartilage and subchondral bone. Unfortunately the success of this procedure is limited due to a complex mix of surgeon- and patient-related variables. An emerging treatment option involves transplantation of the patients’ own stem cells into the osteochondral lesion site after the micro-fracture procedure. Although autologous treatments of this type are considered safe, efficacy is reliant on the quantity and quality of the patients’ stem cells. These important parameters are largely dependent on the age and health status of the patient, as well as the method used to prepare and administer the cells. This proposal seeks to develop and characterise a sugar polysaccharide therapeutic that can greatly augment and sustain the pro-healing effects of stem cell therapy, with a view to providing surgeons with a novel treatment for osteochondral injuries. Students will have chance to participate in one part or the entire project: 1) Generate a HS variant that binds and activates TGF-β1 (hereby referred to as HS16). 2) Develop a device consisting of a mixture of HA, MSCs and HS16 and determine the optimal formulation capable of enhancing chondrogenesis in vitro and osteochondral repair in a small animal model in vivo. 3) Determine the efficacy of the optimized HA/MSC/HS16 device for the repair of osteochondral defects in the knee joints of pigs. Project Co-I: Prof Simon Cool.
neurogenesis and memory loss during aging based on studies of physiologically aged rodents and non-human primates, as well as premature aging mouse models with telomere dysfunction (a hallmark of aging). Indeed, increased DNA damage is found in the aging human brain and a potential source of DNA damage stems from dysfunctional telomeres (i.e., protective caps at the end of our chromosomes) during aging. We will employ the inducible telomerase deficient (TERTER/ER) mouse model to mimic the aging process (through progressive telomere erosion) and its subsequent “rejuvenation”. The inter-generational crossing of this mouse model leads to the late generation (G5) TERTER/ER mice with dysfunctional telomeres, and compromised NSPC functional reserves; reversible by somatic activation of telomerase in the TERTER/ER model (TERT is activated by 4-hydroxytamoxifen (4-OHT)). We will isolate the NSPC with intact versus eroded telomeres for transcriptomic and epigenomic analyses to identify genes/pathways that correlate with impaired NSPC function. Mechanisms that deteriorate NSPC activity will be identified by their reversal upon 4-OHT treatment and functionally validated based on their ability to rescue impaired self-renewal and multipotency of G5 NSPC in vitro and in vivo. Students can look forward to learn mouse modelling, intracranial injection procedure, various –omics (including RNA-Seq, ChIP-Seq and RRBS), single cell qRT-PCR, NSPC assays, flow cytometry and histological analyses.

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Understanding the molecular mechanisms of cellular plasticity: implications in regeneration and aging

The potential utility of stem cells for regenerative medicine has fuelled intense research, culminating in the landmark discovery showing that somatic cells can be reverted into induced pluripotent stem cells. We are interested to address the means by which chromatin plasticity underlies cellular potency. Using embryonic stem cells, we have uncovered several novel transcriptional regulators that may impact stem cell potency through epigenetic state remodeling. The student will undertake molecular and genetic studies to investigate how these novel regulators modulate the dynamic pluripotency state and the impact on cellular differentiation. The urgency for novel and more effective regenerative medicine technologies is underscored by the escalating rates of chronic diseases in an aging population. The student will be exposed to a myriad of advanced techniques such as CRISRP-Cas9 gene editing, Next-Generation-Sequencing technologies (such as ChIP-seq and its derivatives), and genome-wide screening. The lab also utilizes other experimental models of tissue regeneration and epigenetic reprogramming, such as those that occur in the early mammalian embryos and germline. For more information please see:
Aging is an emerging health issue worldwide. Sarcopenia is commonly related to aging and is a major risk factor for mortality. To date, there is no effective and approved pharmacological intervention to treat sarcopenia. The mechanisms to control skeletal muscle growth during aging is also not well understood. In order to address these questions and discover new therapeutic targets to reverse sarcopenia and strengthen skeletal muscle, my lab is devoted to understand the signaling pathways in skeletal muscle growth. One potential regulator of muscle growth is mechanistic target of rapamycin complex 1. Upon exercise and injury, mTORC1 is acutely activated and induces muscle growth and regeneration. Yet chronic activation of mTORC1 has been documented to be associated with aging-related sarcopenia. This suggests that both the timing and duration control of mTORC1 expression are subject to the strict regulation in muscle cells in order to achieve the maximum benefit for injury-induced repair responses and long-term tissue health. To explore how mTORC1 expression in skeletal cells may contribute to the muscle homeostasis, we utilized genomic approach to analyze which genes are specifically modulating or collaborating with mTORC1 activity in mouse skeletal muscle and how they affected the functional aging. mTORC1 is a crucial regulator of protein homeostasis. Activation of mTORC1 leads to phosphorylation of S6 kinases and 4E-BPs, consequently stimulating translation initiation. This project will use the cutting-edge Ribosome-seq technique to monitor mRNA translation in skeletal muscle and further identify the differentially translated genes regulated by mTORC1 during aging. The candidate genes will be validated in a mouse model of sarcopenia to test therapeutic potential in recovery of the muscle function. A better understanding of mTORC1 pathway would lead to the development of more effective and safe therapies to treat aging-related metabolic diseases, and limiting potential off-target side effects while targeting mTORC1.

Department of Psychological Medicine

Principal Investigator: Dr Feng Lei

Project Title with a brief description: Choral Singing for the Prevention of Dementia: A Randomized Controlled Trial

Aims: To evaluate the efficacy of choral singing in the prevention of...
dementia and examine the underlying mechanisms using Magnetic Resonance Imaging (MRI) technique and a panel of peripheral biomarkers in venous blood and urine. Hypotheses: Choral singing could prevent cognitive decline among community-dwelling elderly who are at high risk of dementia. The underlying neural mechanisms involve the changes in brain structure and function that can be quantified using MRI technique. The changes in cognitive outcomes will be accompanied by observable changes from a panel of carefully selected peripheral biomarkers. Study design: randomized controlled trial. Sample size: 360 Inclusion criteria: (1) Community-living elderly aged 60 years and above, and (2) Subjective cognitive complaints based on self-report, or (3) Objective cognitive impairment based on neuropsychological test scores, or (4) Multiple risk factors of dementia such as family history, depression, etc., and (5) Not demented: Clinical Dementia Rating (CDR) global score=0 Intervention period and follow-ups: 2 years Treatment Arms: (1) Intervention arm: weekly choral singing for 2 years (2) Control arm: weekly general health education and group activities for 2 years Primary Outcome Measures: Changes in cognitive performance: composite cognitive test score. Secondary Outcome Measures: Changes in brain structure and function: MRI Changes in biological markers Depressive symptoms: GDS Anxiety symptoms: GAI Stress: PSS Sleep quality: PSQI Neuropsychiatric symptoms: NPI Changes in Rey Auditory Verbal Learning Test Changes in Digit Span Changes in Block Design Changes in Color Trails Changes in Symbol Digit Modality Changes in Boston Naming Severity of symptoms of dementia: CDR Changes in Mini-Mental State Examination Changes in Montreal Cognitive Assessment This study will provide important data on the efficacy and underlying mechanisms of choral singing in the prevention of dementia. Evidence-based choral singing intervention for dementia prevention will has enormous potential impact for healthcare savings and societal welfare.

**Diet and cognitive health in ageing: a community-based prospective cohort study**

In this research study, we will examine the role of diet and nutrition in promoting cognitive health among community-living elderly Singaporeans. The study will be conducted based on the Diet and Healthy Ageing (DaHA) Study in which detailed information on diet and baseline cognitive function has been collected from a group of community living elderly in Singapore. In the new study, we plan to conduct follow-up assessments of 900 participants aged 60+ at baseline using established cognitive tests and clinical tools to determine their cognitive status. Blood and urine samples will be collected to measure biological markers that will provide scientific explanations for the
expected benefits of certain foods, beverages, and dietary patterns. We also will test the interaction between diet and APOE, a gene that affects the risk of Alzheimer disease and other ageing-related conditions. This research study will be the first study that focuses on Asian diets and cognitive health in elderly and may lead to the application of dietary approaches in preventing cognitive decline. Translations of the research findings into clinical and public health practices will potentially help to promote cognitive health at population level and reduce healthcare costs related to cognitive impairment. Specific Aims: 1. To identify dietary factors that are associated with better cognitive function, slower cognitive decline and reduced incidence of mild and major neurocognitive disorders. 2. To investigate if cognitive benefits of such dietary factors can be mediated partially by reduced oxidative damage and systemic chronic inflammation. 3. To investigate interaction between diet and APOE gene polymorphism in influencing cognitive health in the elderly.

Category: Synthetic Biology

Department of Medicine

Principal Investigator          Project Title with a brief description

Dr Chester Lee Drum            Photon mediated therapeutic modulators

Email                           Electromagnetic radiation is an appealing choice for modulation of biological processes within a living organism. From the historical treatment of cancers through X-ray therapy to the recent advent of optogenetics, the ability of light to permeate living tissues without creating off target effects is well proven. Converting incident radiation to physical work or chemical activity, however, presents a major continuing challenge. Tissue and, in particular blood, have wide absorption ranges and permit relatively narrow bandwidths of electromagnetic radiation to penetrate beneath the skin surface. This project will use a chemical biology approach to implement novel, highly photoisomerizable small molecule sensitizers to convert relevant electromagnetic radiation into cell and protein-specific biological actions. Our major work in this area is currently being prepared for publication and will have many follow-on studies of equally high impact. Disease areas amenable to this approach include cardiovascular, cancer and ophthalmic applications. Our lab has a full time Ph.D. – level chemist, biochemist and spectroscopist to aid a motivated graduate student in project design and completion. If interested, please contact the PI for discussion as the ultimate
experimental design will tightly incorporate the intellectual goals of the student in addition to leveraging the current results within the lab.

**Protein Nanoparticles for Engineered Materials and Translational Therapeutics**

Biologicals are quickly becoming a 270 Billion dollar a year industry. Recently the lab has discovered a novel nanoparticle approach to encapsulating functional proteins and stabilizing them against a wide range of cellular and chemical denaturants. In this project you will use a novel form of protein expression invented by our lab to study protein folding dynamics and novel cellular uptake mechanisms for drug delivery. The project is a continuation of research begun at Massachusetts Institute of Technology and will create nanometer scaled uptake vesicles for the delivery of active protein substrates. Our lab has full translational facilities, including recombinant expression, animal protocols, access to human samples and a full complement of mass spectroscopy analytical equipment. Our lab emphasizes a synergistic culture (currently four graduate students), an excellent and productive setting for an efficient approach to a high impact problem, not bounded by a single technique. Although Prof. Drum was clinically trained as a cardiologist (BWH, Harvard Med School) he is also an award winning structural biologist who is interested in developing talented students into future scientific leaders. You are invited to enquire if interested.

**Category: Other Diseases**

**Department of Anatomy**

**Principal Investigator**  Project Title with a brief description

Prof Ong Wei Yi  Translational Studies on Phytochemicals from South-East Asian Herbs

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Background Clinicanthus nutans (C. nutans) commonly known as Sabah Snake Grass in South-East Asia, is commonly used in folk medicine due to its analgesia, antiviral and anti-inflammatory properties. Our previous findings provided evidence for regulation of cytosolic phospholipase A2 (cPLA2) expression by epigenetic factors (Tan-CSH et. al. Mol Neurobiol 2016 53:3854-3872). cPLA2 catalyses the release of arachidonic acid from cell membrane glycerophospholipids. The subsequent metabolism of arachidonic acid produces proinflammatory mediators, free radicals, and oxidative
stress. Current knowledge More recently, we have examined the effects of C. nutans leaf extracts on epigenetic regulation of cPLA2 expression. C. nutans modulated induction of cPLA2 expression in SH-SY5Y cells and reduced damage to primary cortical neurons subjected to oxygen glucose deprivation (Tan-CSH et al. Neuromolecular Medicine 2016 18:441-452) Gaps which project is intended to fill The above studies on C. nutans have been carried out in vitro. The aim of the present project is to translate the findings to animal models of disease, e.g. chronic inflammation, sleep apnea, stroke, etc. for possible clinical use. Phytochemicals from other South East Asian herbs will also be explored. Students who are interested in how basic knowledge on phytochemicals could be translated to clinical use are invited to apply.

Department of Biochemistry

Principal Investigator

Dr Long Yun Chau

Project Title with a brief description

Role of insulin-induced glycolysis in the regulation of PGC1α-mediated myotube lipid metabolism.

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Our body is subjected to irregular nutrient supply, including the transition between fasting and feeding. Therefore, the ability of our body to selectively store and utilize different energy substrates is critical for energy balance. Given the substantial mass and energy consumption, skeletal muscle plays a critical role in the regulation of energy balance, and impaired skeletal muscle metabolism is closely associated with metabolic diseases such as type 2 diabetes and obesity. Thus, investigation of skeletal muscle energy substrate metabolism is critical for the understanding of pathophysiology of metabolic diseases. Under postprandial conditions, insulin stimulates skeletal muscle glucose uptake and utilization via Akt, a protein kinase that activates downstream effectors of insulin. Conversely, skeletal muscle increases lipid oxidation under fasting conditions, which coincident with the induction of PGC1α - a transcription coactivator that has been implicated in the induction of lipid metabolic gene program. Nonetheless, the interaction between the disparate insulin and PGC1α pathways which are activated under contrasting nutritional states remains largely unknown. This research project aims to establish the role of nutrient (glucose) and hormone (insulin) in the regulation of PGC1α cultured myotubes, and the impact of this regulation on lipid metabolic gene program in myotubes.

Role of insulin-like signaling in the regulation of SIRT1 deacetylase
The loss of muscle function in diseased state is attributed to elevated protein degradation and resistance to anabolic effects of growth hormones. The anabolic hormones are critical signals that stimulate skeletal muscle glycogen and protein synthesis (anabolic metabolism), predominantly via the Akt kinase pathway. Conversely, nutrient deprivation activates Silent Information Regulator T1 (SIRT1) deacetylase and forkhead box protein O1 (FOXO1) proteins that induce catabolic metabolism in skeletal muscle. The increase of catabolic over anabolic pathway may be a molecular basis for the loss of muscle mass and function. Nonetheless, the mechanism by which such delicate balance is regulated remains largely unknown. This proposal aims to evaluate the novel role of insulin-like signals in the regulation of SIRT1, and the unknown function of SIRT1 in the regulation of glucose and amino acid metabolism in cultured myotubes. The specific aims are (1) to determine if insulin-like signals regulate SIRT1 via nutrient metabolism, (2) to establish the role Akt in the regulation of SIRT1-induced FOXO1 pathways, and (3) to examine the impact of AKT-SIRT1 pathways on energy substrate metabolism. Cultured myotubes will be treated with insulin or IGF1 in the absence of presence of metabolic inhibitors to determine the function of these hormones on SIRT1 function. The role of Akt in the regulation of SIRT1-dependent FOXO1 activity will be evaluated via pharmacological and genetic alteration of the kinase and deacetylase. The metabolic effects of Akt-SIRT1 pathway will be determined by direct measurements of glucose and amino acids metabolism in myotubes to provide direct readout for changes in metabolic fluxes. The research will provide important insight into the pathophysiology of skeletal muscle metabolism, via the identification of insulin-like hormones as regulators of SIRT1. It will also provide critical evidence for SIRT1 and IGF1 as potential drug targets for muscle wasting diseases.

The role of energy sensing network in exercise-induced skeletal muscle adaptation

The beneficial effects of exercise on health are widely recognized. Exercise could improve and prevent chronic metabolic disorders such as diabetes and obesity. However, the cellular mechanism behind these effects is largely unknown. Optimization of exercise regimen or pharmacological recapitulation of exercise effects requires close examination of intramuscular events that triggers the exercise adaptations. Our previous and current work has aimed to define the mechanism that drives exercise-induced metabolic adaptation in skeletal muscle. Exercise induces a multitude of cellular disturbances in muscle including decreased intramuscular energy levels. Exercise-
induced energy deficit is considered an important stimulus for the beneficial metabolic outcomes. AMP-activated protein kinase (AMPK) is an important signal transducer which is activated in response to energy stress during exercise. Our previous work in mouse models provided evidence that activation of this protein kinase is sufficient to increase skeletal muscle glycogen store, fatty acid utilization and muscle endurance – benefits that are derived from exercise training. Conversely, disruption of muscle AMPK signaling accelerated the progression of muscle fatigue and impaired exercise-mediated metabolic gene expression. These results provided important evidence that AMPK signaling mediates some of the exercise-mediated metabolic responses in skeletal muscle. In the current study, we hypothesize that AMPK plays a role in autophagy and amino acids metabolism in skeletal muscle, and this effect is critical for metabolic benefits of exercise and endurance.

Department of Medicine

Principal Investigator          Project Title with a brief description

Dr Chester Lee Drum        Deep Learning (AI) Approach to Cancer Treatment Risk Prediction

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Deep learning is a subset of machine learning which uses very deep neural networks to learn complex relationship between input variables and final output in very large datasets. In a process called supervised learning, an algorithm is trained iteratively on a high-quality annotated dataset. Each iteration culminates with a hypothetical/predicted output which is compared against the real output and the error is used intelligently to adjust the weight associated with each input parameter for the next iteration and the process is continued till the predicted output matches the real output. Finally, the trained model is used to predict the outcome for a new set of inputs. Working with bioinformaticians, clinicians and large cardiovascular and cancer data sets, you will create novel algorithms to identify patients at risk of drug adverse effects and who will benefit from a given treatment. You will be listed as an official member of the ethics approval and work with patient data to improve medical outcomes. A robust model of immediate translational value will be created to predict adverse reactions. In particular, one of the largest datasets of individual patients with quantified drug levels and characterized genomic data will serve as a training template for a first of its kind prediction algorithm that warns patients of adverse outcome and helps them determine the correct dosing. There is also a potential for collaboration with Singapore Super Computing Centre based on candidate needs and
Understanding dendritic cell and B cell sensing of autologous cellular antigens and antinuclear autoimmunity

Intracellular antigens are generally tolerated by autologous immune cells and, where this tolerance is breached, autoimmune diseases are caused. In live cells, intracellular antigens are privileged from direct recognition by immune cells, but they can become exposed during cell death. Cells that die from apoptosis are often rapidly cleared by phagocytes before leakage or exposure of intracellular antigens without inducing immune responses. However, cells that die following the necrotic pathway expose these antigens which can activate autoreactive B cells through the B cell antigen receptors (BCR) and activate antigen-presenting cells, such as dendritic cells, through intracellular alarmins or DAMPs, which then activate autoreactive T cells. Precisely, how B cells and dendritic cells respond to necrotic cellular molecules remain poorly understood. In this project, we examine the roles of Colec9A and Clec12A, which are dead cell receptors expressed on both B cells and dendritic cells, in the responses to intracellular antigens and alarmins. Project anticipates experiments with fluorescence imaging, dendritic cell and B cell activation assays, ELISA/multiplex cytokine assays, flow cytometry, proteomics, etc. The target disease for this project is systemic lupus erythematosus (SLE). SLE patients develop autoantibodies against autologous nuclear antigens to cause tissue inflammation and injuries. This disease affects a large number of young women and has no specific therapeutic treatments.

Exploring novel mitochondrial delivery vectors for mitochondrial gene therapy

Defects of all protein-coding mitochondrial genes have been associated with human, mainly neurodegenerative disorders or with aging. Mitochondrial gene therapy, however, is hampered by the lack of an efficient mitochondrial gene delivery system. Recently, we developed a novel scalable mitochondrial targeting vector which is based on RNA subdomains of a long non-coding RNA derived from the human cytomegalovirus. We demonstrated that this vector system can efficiently target functional recombinant coding or non-coding RNA to the mitochondria resulting in mitochondrial expression of recombinant
RNA sequences or knock-down of mitochondrial gene expression. The aim of this project is to further optimize and study the mitochondrial delivery vector system and to investigate as to whether this vector system is suitable to deliver complete mitochondrial genomes into the mitochondria. A second strategy will be to deliver the CRISPR/Cas genome editing technology into mitochondria in order to trigger the selective destruction of defect mitochondrial genes. This project is highly translational and can be explored towards mitochondrial gene therapy of yet incurable human diseases.

**Department of Orthopaedic Surgery**

**Principal Investigator**  
Prof Lee Eng Hin  

**Project Title with a brief description**  
**Engineering MSC Paracrine Signaling for Tissue Regeneration**

Mesenchymal stem cells (MSCs) hold significant promise for tissue engineering and regenerative medicine due to their relative ease in isolation from various human tissues, their unique properties such as their ability for proliferation, their capacity for multilineage differentiation, their immune privileged status and the lack of ethical concerns in their use. More recently there has been increasing interest shown in the therapeutic effects of MSCs through their paracrine signaling. It is known that MSCs secrete a plethora of bioactive factors such as cytokines, chemokines, growth factors, miRNAs that can elicit a host of biological responses in recipient cells leading to amplification of the regenerative response. It is proposed that these secreted active biomolecules can modify the immune response and enhance the regeneration of injured tissues. An area of interest is how the paracrine factor production and release by MSCs can be modulated and directed to specific regenerative applications. Cues from the extracellular environment and physical stimuli are known to affect MSC proliferation and differentiation. In this study, various strategies to manipulate the cellular microenvironment will be explored to control the MSC secretome for musculoskeletal tissue regeneration. This study will include the induction mechanism and characterisation of the induced secretome that affects specific functional outcomes. The student will leverage on skills in scaffold fabrication, surface modification, cell culture, histology, microarray and proteomic analysis. This project will be carried out with the collaboration of A/P Alfredo Franco-Obregon from the Department of Surgery.

**Department of Paediatrics**
Immunological profiling of paediatric patients with focal segmental glomerulosclerosis responsive to rituximab therapy: Linking the synapses and defining the key players

Focal segmental glomerulosclerosis (FSGS) is an important cause of chronic kidney disease in children and adults. In recent years, case reports have emerged regarding the successful induction of remission in patients with FSGS using rituximab who have otherwise failed therapy with steroids and these second-line immunosuppressive drugs. This suggested that B-cells may play a role in the pathogenesis of FSGS, either directly or indirectly through B-T interactions, in some patients with FSGS. In our cohort of FSGS patients receiving rituximab treatment, we showed that a specific immunological profile characterised by hyporesponsive T-cell to activation (HT) was able to predict rituximab response. We therefore postulate that in this subgroup of patients with immune-mediated FSGS, T-cells are important in the regulation of an immune cascade that leads to the production of a putative permeability factor ultimately resulting in proteinuria. Our preliminary experiments suggest that defective B-cell regulation could result in hyporesponsiveness of T-cells which are then unable to limit monocyte/macrophage production of a permeability factor(s). Rituximab depletes B-cells which subsequently restores T-cell responsiveness to activation, explaining its efficacy in these patients. The main objective of this project is therefore to delineate the pathogenetic pathways leading to nephrotic syndrome in this particular subgroup of immune-mediated FSGS with T-cell hyporesponsive to activation (HT) by (1) phenotyping the T-cell and B-cell subsets, (2) investigating the nature of the B-T interactions and (3) studying the defective regulation of T-cells on monocyte/macrophage production of putative permeability factor(s) in order to elucidate both the upstream and downstream immune effectors. The candidate permeability factor(s) will be studied by overexpressing the factor(s) in an animal model (Wistar rats) in order to reproduce the proteinuric phenotype with histological features consistent with FSGS.
Inflammation in Asthma, COPD and Lung Fibrosis

This laboratory is funded to study different mechanistic aspects of respiratory diseases: (1) The impact of allergens on DNA damage and repair in asthma, and on LysRS-Ap4A-MITF signaling pathway- and p38 MAPK signaling pathway-mediated airway inflammation. Allergens can directly damage airway epithelium by increasing free radicals and DNA damage. Strategies to mitigate free radical levels and block DNA damage may alleviate allergic asthma. The LysRS-Ap4A-MITF pathway plays a critical role in mast cell degranulation and activation, which contribute to the development of allergic asthma. Efforts to regulate this pathway may provide novel therapeutic targets for asthma therapy. My collaborator is constructing p38 MAPK transgenic mice and we will study the constitutively active p38 MAPK in asthma development. (2) We have a cigarette smoke-induced COPD mouse model and are now developing PM 2.5 pollutant-induced COPD model. We are testing novel molecules to protect and treat COPD. In addition, COPD is insensitive to corticosteroid treatment, and we are developing pharmacological strategy to reverse the steroid-resistance process. (3) We have a robust bleomycin-induced idiopathic pulmonary fibrosis (IPF) model. IPF patients have only 5 year mean-survival rate after diagnosis with limited treatment options. We are testing new molecules for the treatment of IPF. Publications: Chan TK, et al. (2016) House dust mite-induced asthma causes oxidative damage and DNA double-strand breaks in the lungs. J Allergy Clin Immunol 138:84-96. [IF=12.485] J. Dong, et al. (2017) Ribosomal protein S3 gene silencing controls house dust mite-induced experimental asthma. Br. J. Pharmacol. 174:540-552. [IF=5.259] W. Liao, et al. (2016) Andrographolide restores steroid sensitivity to block LPS/IFNg-induced IL-27 and airway hyperresponsiveness in mice. J Immunol. 196:4706-4712. [IF=6.293]