SHERRINGTON TALKS 2021 ONLINE DAY ONE

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS
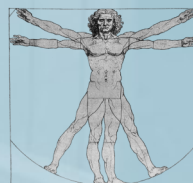
JOIN MICROSOFT TEAMS MEETING

FRIDAY 11TH JUNE

1PM

Presented by DPAG Graduate Students in their 3rd year of DPhil research study

Chaired by Deputy DGS A/Professor Vladyslav Vyazovskiy

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

UNIVERSITY OF OXFORD

SHERRINGTON TALKS 2021

Judges

Graduate Studies Committee Academics and the audience

Prize winners will be notified in the Digest on Monday 21 June

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

Tanadet Will Pipatpolkai

From clinical to computational : the story of PIP2 and neonatal diabetes

Supervisors: Professor Dame Frances Ashcroft & Professor Phillip Stansfield

Membrane proteins are frequently modulated by specific protein-lipid interactions in the phospholipid bilayer. The activation of ATP-sensitive potassium (KATP) channel by phosphatidylinositol-4,5-bisphosphate (PIP2) has been well characterised experimentally, with differences in the extent of channel activations. Here, we establish a coarse-grained molecular dynamics free-energy perturbation (CG-MD-FEP) methodology to capture the effect of neonatal diabetes mutation to the binding of PIP2 lipids to Kir6.2 channels. By perturbing amino acid side chains on Kir6.2, we show that neonatal diabetes mutation (E179K) has increases PIP2 affinity, while a congenital hyperinsulinism mutation (K67N) results in a reduced affinity. This is in strong agreement with our electrophysiological studies where the E179K mutant exhibits a reduction in neomycin sensitivity and ATP sensitivity. To conclude, the methodology provides a platform of annotating amino acid side chains within lipid-binding pockets and their role in disease.

Lucija Fleisinger

Endothelial *KLF2* is developmentally regulated by two distal enhancers

Supervisors: Associate Professor Sarah De Val & Dr Alice Neal

The development of a functional vascular system is critically dependent on shear stress, a hemodynamic force exerted onto the vascular endothelium by flowing blood. Although one of the key effects of shear stress on endothelial cells is modulation of gene expression, our understanding of transcriptional networks governing endothelial responses to shear stress is incomplete.

The *KLF2* gene encodes a shear stress-responsive transcription factor that is robustly expressed in both the developing and adult vascular endothelium. Deletion of *KLF2* during vascular development results in embryonic lethality, whilst conditional *KLF2* deletion in the adult endothelium worsens atherosclerotic plaque burden in mouse models. Despite this crucial role in maintaining a functional endothelium, the mechanisms by which shear stress regulates *KLF2* expression remain controversial. Activation of *KLF2* by shear stress was previously hypothesised to occur through a MEK5-ERK5-MEF2 pathway thought to interact directly with the *KLF2* promoter, thereby inducing *KLF2* expression. However, MEF2 factors are pan-endothelial effectors of signalling pathways not responsive to shear stress. Further, this promoter-centric model was never validated in vivo, and the role of distal enhancer elements in the transcriptional regulation of *KLF2* has never been investigated.

Here we show that endothelial *KLF2* is transcriptionally regulated by two distal enhancers independently of its core promoter during development. Each *KLF2* enhancer directs a distinct pattern of endothelial-specific reporter gene expression corresponding to expression of the endogenous *KLF2*. Early results from pharmacological manipulation of blood flow in vivo suggest that reporter gene expression driven by these enhancers changes in response to shear stress. Phylogenetic foot printing combined with mutational analysis of these *KLF2* enhancers has identified a functional MEF2 binding motif adjacent to a second, yet to be identified, motif, both of which are required for enhancer activity. This work demonstrates previously unappreciated complexity in the transcriptional regulation of *KLF2*, and provides an opportunity to better understand transcriptional dynamics at the locus of this disease-relevant gene.

Ujang Purnama

Modelling the human diabetic heart, using engineered heart tissue, for in-vitro testing of cardioprotective drugs

Supervisors: A/Professor Carolyn Carr, A/Professor Lisa Heather & Professor Kim Dora

Aim: Diabetes is a global epidemic, with cardiovascular disease the leading cause of death in diabetic patients. There is a pressing need for an *in-vitro* model to aid understanding of the factors of the diabetic phenotype that are harmful to cardiac tissue and to provide an accurate, predictive tool for drug testing. Human induced-pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have potential as a predictive tool but are hindered by their immature phenotype. 3D culture of hiPSC-CMs as engineered heart tissue (EHT) is the most advanced approach to mature the cells. Here, we show for the first time how to modulate the media to mature cells in the EHT and cause them to become insulin resistant.

Methods and Results: Culturing EHT in media containing oleic acid resulted in maturation of sarcomere structure, action potential, gene expression, and a metabolic switch to fatty acid oxidation, recapitulating more mature cardiomyocytes. We developed a diabetic medium by modulating levels of glucose, insulin and palmitic acid which induced an increase in fatty acid metabolism and a blunted insulin response, indicating that the hiPSC-CMs had become insulin resistant (IR). We subjected IR-EHT to hypoxia and adrenergic stimulation and measured contractility using Muscle Motion. We saw a metabolic inflexibility of the cells under hypoxia which mimicked that seen in diabetic rat hearts. Treatment of IR-EHT with Molidustat (BAY85-3934), a prolyl hydroxylase (PHD) inhibitor, stabilised Hypoxia-Inducible Factor 1-α signalling in hiPSC-CMs and increased glycolysis, thereby validating its potential use as a therapeutic in the diabetic heart.

Conclusion: Our results show that we have successfully generated a clinically relevant *in vitro* model of insulin-resistant human heart tissue to study the pathophysiological effects of diabetes. This can be a valuable predictive tool to speed up the drug discovery process and improve candidate drug success in clinical trials.

Dr Peregrine Green

Metabolic Determinants of Left Ventricular Reverse Remodelling in heart failure

Supervisors: A/Professor Neil Herring & A/Professor Oliver Rider

Background: Cardiac resynchronisation therapy (CRT) is a well-established therapy for treatment of severe heart failure with reduced ejection fraction (HFrEF) with left bundle branch block (LBBB), resulting in varying degrees of reverse remodelling and improvement in LV systolic function, although this process is poorly understood. There is increasing evidence that reduced cardiac metabolic flux in heart failure may drive decreased contractility, but whether this also limits the ability of the heart to reverse remodel in response to CRT is unknown.

Aims: In patients with HFrEF put forward for CRT, I aim to take measurements of metabolic flux using cardiac magnetic resonance (MR) imaging and 31P-spectroscopy, and then use coronary blood flow, blood sampling and pressure-volume loop measurements during CRT implantation to calculate acute metabolic substrate uptake and use by the heart in response to CRT. This is then compared to the degree of LV reverse remodelling using cardiac MR imaging 6 months following CRT implantation.

Results: 10 patients (6 male, mean age 65 +/- 7 years) have been recruited to date for MRI and invasive measurements during CRT. CRT acutely increases contractility measured as the rate of rise in left ventricular pressure (dP/dt 641 ± 125 vs 712 ± 136 mmHg/s (mean ± SD), p = 0.0029) with a trend towards increased stroke work (mean 8483 ± 2874 vs 10955 ± 916 ml.mmHg, p = 0.12) despite no change in myocardial oxygen uptake (MVO2 14.6± 9.1 vs 13.6 ± 8.9 ml/min). There was a trend towards increased free fatty acid uptake (-0.005 ± 0.02 mmol/min vs 0.039 ± 0.07, p = 0.06) but no change in glucose uptake (0.071 ± 0.029 vs 0.081 ± 0.068 mmol/min, p = 0.65). Similar results were seen during stress measurements at 65% predicted maximum heart rate.

Conclusion: Preliminary results indicate that CRT increases cardiac efficiency, and that this may be associated with increased free fatty acid uptake but not glucose uptake. Whether the ability to increase fatty acid uptake, or other aspects of cardiac metabolism (such as the size of the phosphocreatine pool, or ability to increase creatinine kinase flux) are associated with the degree of reverse remodelling is yet to be determined.

William Stockdale

Myocardial proliferation and the metabolic response duringheart regeneration in the Mexican cavefish

Supervisors: A/Professor Mathilda Mommersteeg & Professor Paul Riley

Following myocardial infarction, the human heart is unable to repair itself and forms a permanent scar that impairs cardiac function and may later result in heart failure. The zebrafish, amongst other organisms, has been extensively studied for its innate ability to repair its heart after injury, with studies indicating myocardial proliferation as a key driver to the regenerative process.

We have established the *Astyanax mexicanus* as a unique model for cardiac regeneration, which allows us to compare adult regeneration with human-like scarring within a single species. In this model the *Astyanax* surface fish regenerate their heart after injury, while their cave-dwelling counterparts cannot and, similar to humans, form a permanent scar.

Investigation of proliferation in the *Astyanax* identified myocardial proliferation peaking to similar levels in both surface fish and cavefish 1 week after injury in the region adjacent to the wound. However, a BrdU pulse chase experiment revealed cavefish cardiomyocytes do not complete cellular division and ultimately fail to replace the lost myocardium. To understand the mechanisms that underlie this proliferation profile, bulk RNA-seq and single cell RNA-seq datasets of *Astyanax* hearts after injury were analysed. These revealed strong expression differences relating to the metabolism between cavefish and surface fish hearts, with single cell RNA-seq determining metabolic differences specific to the proliferating cardiomyocytes. Further validation specifically found glycolysis related genes highly upregulated in the surface fish heart but not in the cavefish after injury, and interestingly, expression of these genes are localised to the wound border where myocardial proliferation takes place – suggesting an importance of glucose metabolism in cell cycle and heart regeneration.

Investigating the metabolic response in the *Astyanax mexicanus* after injury will help define the ideal metabolic environment for complete myocardial proliferation and successful heart regeneration.

Louisa Zolkiewski

Investigating the role of TBX15 in adipogenesis

L Zolkiewski[I], R Dumbell[I, II], E Bentley[I], M Simon[III], R D Cox[I].

I. Genetics of type 2 Diabetes, MRC Harwell Institute, Harwell Campus, OX11 0RD, UK; II. School of Science and Technology, Nottingham Trent University, Nottingham, NG1 4FQ, UK; III. Bioinformatics, MRC Harwell Institute, Harwell Campus, OX11 0RD, UK

Supervisors: Professor Roger Cox & Professor Clive Wilson

Elevated waist-hip ratio (WHR) increases the risk of type 2 diabetes and cardiovascular disease. The most recent genome-wide association studies have identified over 463 signals in 346 loci associated with WHR adjusted for BMI (WHRadjBMI). Within the *TBX15-WARS2* gene locus several independent association signals marked by single nucleotide polymorphisms (SNPs) have been associated with WHRadjBMI, following conditional analyses. Studies, *in vitro* and *in vivo*, indicate that *Tbx15* plays a role in adipogenesis, although the precise mechanisms are unclear. We hypothesize that TBX15 regulates transcription of a network of genes relevant to adipogenesis and fat distribution. In order to investigate the physiological effect of *Tbx15* on fat distribution, heterozygous knock-out ($Tbx15^{+/-}$) and wild-type ($Tbx15^{+/+}$) mice were challenged with a high fat or low fat diet from weaning. Metabolic phenotyping studies were then initiated to evaluate overall growth, glucose and insulin sensitivity, and fat distribution to 24 weeks of age. Preliminary echo-MRI data analysis using 2-way ANOVA indicated an overall genotype ($p=0.0311$) and time x genotype ($p<0.0001$) effect for body weight in female mice on a low fat diet. $Tbx15^{+/-}$ mice were significantly smaller than $Tbx15^{+/+}$ littermates at several time points ($q<0.05$, multiple unpaired t-tests). This difference was reflected in total fat mass values at 16 and 18 weeks (nominal $p<0.05$, multiple unpaired t-tests). In order to evaluate the effect of this transcription factor on gene expression in adipose tissue, subcutaneous and gonadal white adipose and brown adipose tissues were isolated from $Tbx15^{-/-}$ (*Tbx15* null) and $Tbx15^{+/+}$ mice for RNA sequencing. Preliminary analysis indicated 2040 genes were significantly differentially expressed between $Tbx15^{+/+}$ and $Tbx15^{-/-}$ mice in subcutaneous white adipose tissue ($q<0.05$). gProfiler pathway analysis indicates genes downregulated ($n=1713$) in $Tbx15^{-/-}$ mice are associated with immune receptor activity, whilst upregulated genes ($n=327$) were associated with muscle functions. Further work is underway to further validate and investigate these differences *in vitro* and *in vivo*.

Andrew Shelton

Investigating the organizing principles of the mouse claustrum

Supervisors: Dr Adam Packer & A/Professor Simon Butt

Background: The claustrum is the most densely interconnected region in the mammalian brain. Previous research has demonstrated that the claustrum is active in a variety of contexts and has been implicated in sensory integration, attention, pain sensation, sleep, and saliency detection. However, it remains unclear what the precise function of the claustrum is or how this relates to its vast connectivity. A notable impediment is the lack of information regarding how individual claustrum neurons respond to input from the many cortical areas that project there or how these inputs are spatially organized within the claustrum itself. By understanding these organizing principles, we hope to provide a framework for future studies that wish to address claustrum function in the context of behavior.

Aims: We seek to assess claustrum circuitry at the single cell level in order to determine the physiological and anatomical relationship claustrum neurons have with multiple upstream cortical areas.

Methods: We use dual-color optogenetics, whole cell patch clamp electrophysiology, and immunohistochemistry to specifically label, record from, and perturb claustrum neurons of the mouse.

Results & Conclusions: Our study has so far revealed a highly specific synaptic architecture that integrates and routes information from cortical regions associated with cognition and executive control through the claustrum to distant and disparate areas around the brain. We have also demonstrated that the claustrum is arranged into modules that can be defined both physiologically and anatomically. These insights furnish a growing body of work aiming to address claustrum activity globally by demonstrating important local organizing principles.

Carla Martin

Title Protein features for cargo sorting to extracellular vesicles (EVs)

Supervisors: Dr Thomas Roberts, Dr Imre Mager & Professor Matthew Wood

Aim: To identify protein features promoting EV sorting for therapeutic cargo loading to EVs and to determine their biological role in EV sorting.

Methods and Results: A bioinformatics program was developed to find candidate EV-loading features from HEK293T, MSC and adipocyte cells and EV proteomics data. Several protein features (post-translational modifications, domains and motifs) were selected and assessed for their EV-loading capacity using GFP fusion constructs in HEK cells. Proteins containing the given feature were fused to GFP for their assessment. EVs were isolated using tangential flow filtration followed by size-exclusion chromatography. EV-enrichment was tested by western blot and fluorescence of the EV fractions derived from cells expressing the GFP-fusion constructs. N-linked glycosylation on PTTG1IP protein was found to be the feature with the highest EV-loading capacity *in vitro* and therefore was studied in more detail. Non-glycosylated PTTG1IP mutants were generated to confirm the presence of the N-glycosylation sites and to determine the effect of N-glycosylation on EV-loading. A marked decrease in EV-enrichment was found in the mutants as compared to the fully glycosylated protein.

Conclusion: The bioinformatics analysis we have developed has enabled the identification of several EV-loading protein features of which N-glycosylation has been found to be the most efficient. N-glycosylated PTTG1IP has been shown to enable efficient sorting to the EVs and its N-glycosylation was critical for its loading into the EVs.

Sonali Munshaw

Thymosin β4 protects against aortic aneurysm via endocytic regulation of growth factor signalling

Supervisors: A/Professor Nicola Smart & Professor Paul Riley

Introduction: Aortic aneurysm (AA) is a degenerative vascular disease and a leading cause of mortality. Dysregulation of smooth muscle cell (VSMC) phenotype critically impairs vascular stability. Low density lipoprotein receptor related protein 1 (LRP1), an endocytic regulator of VSMC PDGFRβ signalling, is associated by GWAS with AA risk. Thymosin β4 (Tβ4) is an actin binding peptide required for embryonic VSMC differentiation. We validated a novel interaction between Tβ4 and LRP1 in VSMCs. As a regulator of VSMC differentiation, we hypothesise that Tβ4 interacts with LRP1, to maintain healthy vasculature postnatally and protect against disease.

Objectives:
- To determine whether Tβ4 knockout mice display increased susceptibility to AA.
- To elucidate the molecular mechanism of growth factor signalling via Tβ4 –LRP1.
- To explore the therapeutic potential of exogenous Tβ4 in vascular protection.

Methodology: Global and VSMC-specific Tβ4KO mice were infused, alongside controls, with 1mg/kg/day Angiotensin II. For rescue experiments, C57BL/6 mice additionally received 12mg/kg/day Tb4 or saline. VSMC phenotype, signalling, elastin integrity, ECM composition, and inflammation were analysed using FACS, histology, immunostaining, and immunoblotting. Using primary VSMCs and MOVAS-1 cells, endocytosis of the LRP1-PDGFRβ complex was tracked by surface biotinylation and proximity ligation assays, following PDGF-B stimulation.

Results: Global and VSMC-specific Tβ4KO mice, like LRP1 KO, demonstrated predisposition to AA, with aortic dilatation and rupture in <5 days. Accelerated disease progression was not caused by exacerbated inflammation, rather by enhanced VSMC phenotypic switching and dysregulated LRP1/PDGFRβ signalling. Tβ4-depleted VSMCs demonstrated increased recycling of LRP1-PDGFRb to the plasma membrane and reduced lysosomal targeting, following PDGF-B stimulation. Exogenous Tβ4 significantly reduced aortic dilatation and rupture, with preserved VSMC and elastin phenotype and normalised PDGFRβ signalling.

Conclusion: We identify Tβ4 as a key regulator of LRP1-PDGFRβ endocytic signalling, for maintaining VSMC differentiation and vascular health. Tβ4 is a promising candidate for treatment of vascular disease.

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