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Molecules, Cells and the Basis for Disease

This theme brings together stem cells and regenerative medicine (inc. cellular therapies), immunology, genetics, cellular biology (particularly relating to cancer), and biophysics. These areas – and particularly the interfaces between them – are current strengths and priorities for King’s.

Lead: Professor Rebecca Oakey

Projects beginning with K are KBI projects for example K1.1
1.1 Membrane remodelling during cell division

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Project description:

During division, cells undergo dramatic remodelling of their membranes and cytoskeleton. Whilst we know much about the proteins involved in this remodelling, our understanding of the role of lipids in this process remains severely underappreciated. Faithful cell division is essential for the maintenance of genome integrity and failures in this process are thought to underlie a variety of human malignancies.

We will focus on a membrane-remodelling complex called the ESCRT-machinery. The ESCRT-machinery is essential for cytokinesis and reformation of the nuclear envelope during mitotic exit and additionally drives cargo sorting on endosomes and release of enveloped retroviruses such as HIV-1. We know the ESCRT-machinery can remodel membranes, but we don’t know how this machinery interacts with membranes.

You will use genome-editing to tags endogenous ESCRT-components and will develop protocols to allow stabilisation of these proteins on cellular membranes during mitosis. You will extract the tagged ESCRT-components and will perform mass spectrometry and lipidomic analysis to identify lipid species bound. Once candidate lipids have been identified, you will verify interactions using liposome-based binding assays. By depleting or removing enzymes necessary for production of the lipid species in question, you will test whether ESCRT components fail to localise to their sites of action and will analyse the consequences of this failure for cell division.

You will join laboratories examining the molecular control of cell division (Carlton laboratory – ESCRTs; Eggert laboratory - lipids and the cytoskeleton) and will be trained in techniques including molecular biology, advanced imaging, protein biochemistry, lipidomics and lipid biochemistry.

Two representative publications from supervisors:


2.1 The nanomechanics of the LINC complex-from the single molecule to the single cell.

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Project description:
There is now mounting experimental evidence suggesting the intriguing possibility that mechanical forces at
the cell surface may promote mechanochemical conversion in the nucleus. Considering the large distance
between the cell wall and the nucleus, transmission of the force signal by simple chemical diffusion would result
in a slow and inefficient process. Rather, the emerging alternative model suggests that the mechanical impulse
is propagated through a “hard-wire” protein network that links the cell membrane with the nuclear envelope,
through the cytoskeleton. The LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, formed by the
association of SUN and nesprin proteins, allows a physical connection between the intermediate filament/actin
cytoskeleton and the nucleoplasm. It is very tempting to speculate that these external mechanical forces could
affect the mechanical stability of the cell nucleus and modulate the nuclear shape, with consequent
conformational changes in the chromatin structure and organization.

In this project we will investigate the effect of each of the LINC complex partners on the mechanical properties
of individual live cells and isolated nucleus. The student will gain expertise in single molecule and single cell
Atomic Force Microscopy characterisation, combined with cell and molecular biology techniques. It is
expected that in Year 1 cell biology experiments will be performed at CS lab. Year 2 will be devoted to
conduct single molecule and single cell mechanical experiments using AFM (SGM lab). Experiments and
analysis will continue in Year 3.

This is a unique opportunity to explore fundamental physical questions underlying the function of the LINC
Complex in cells, combining expertise in cutting-edge mechanical biophysical techniques (Garcia-Manyes)
and modern cell biology techniques (Shanahan).

Two representative publications from supervisors:

in a bimolecular chemical reaction», Nature Chemistry, (2009), 3, 236-242

DT, Wheeler MA, Ellis JA, Skepper JN, Vorgerd M, Schlotter-Weigel B, Weissberg PL, Roberts RG,
Wehnert M, Shanahan CM. (2007) Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss
muscular dystrophy and are critical for nuclear envelope integrity. Hum Mol Genet. 16(23):2816-33.

between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus
3.1 Control of amyloid precursor protein trafficking by kinesin-1

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Project description:  
By virtue of its capacity to transport many intracellular cargoes, the microtubule motor kinesin-1 plays a central role in neurological, viral and bacterial diseases, as well as in cancer cell metastasis. One key kinesin-1 cargo is amyloid precursor protein (APP) that, when proteolytically processed, forms beta-amyloid plaques that are thought to play a central role in the pathology of Alzheimer’s disease (AD). Mis-regulation of kinesin-1 has also recently been shown to play a causative role in AD. At present, we lack a good understanding of the molecular mechanisms that govern neuronal kinesin-1-dependent transport of APP. A better understanding of these mechanisms could lead to the development of new therapeutic approaches. Combining the specialist skills of both co-supervisors we have recently solved the first crystal structure of a kinesin-1:cargo complex providing the structural basis for the recognition process for cargoes containing tryptophan-acid (W-Ac) motif(s), which in themselves are important in this process. We are therefore in an excellent position to apply a combination of cutting-edge structural (nanobody-mediated crystallization)(years 1/2), biophysical and cell-based approaches (high speed, high resolution live cell imaging and FLIM-FRET)(years 3/4) to further define the molecular basis of transport of APP, focusing on the basis of cargo recognition and regulation for the APP transport by the adaptor protein JIP1 as well as other candidates. We will explore how these molecules couple APP to kinesin-1 and how they regulate the activity of the motor.

Two representative publications from supervisors:


4.1 Effects of ambient diesel carcinogens on pulmonary inflammation and DNA damage

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Project description:
Exposure to diesel exhaust is causally associated with several chronic respiratory diseases, including lung cancer, asthma and chronic obstructive pulmonary disease (COPD), yet the mechanism of diesel carcinogenesis and precise identity of the critical carcinogenic components of diesel exhaust are unclear. This project aims to elucidate the role of inflammation in respiratory tract DNA damage associated with exposure to ambient air and diesel particulate matter (PM), collected at multiple locations in central London differing in the extent of ambient diesel emissions or at an engine/fuel testing facility. Toxic chemicals bound to PM include polycyclic aromatic hydrocarbons (PAHs), many of which are highly toxic and/or carcinogenic. The specific aims of the project are: 1) To develop a multi-cellular 3D in vitro model that mimics the human airways and can be used to elucidate how neutrophils and macrophages impact on the pulmonary bioactivation of ambient diesel carcinogens and how ‘activated’ metabolites promote DNA damage and inflammation in lung epithelial cells. 2) To understand the role of inflammation on promoting respiratory tract DNA damage and mutagenesis after exposure to exposure to ambient diesel carcinogens in vivo using a transgenic mouse mutation assay and to investigate the impact of pulmonary neutrophil influx in vivo on tissue-specific effects related to toxicity and tumour development (cytokine/chemokine expression, global gene expression, inhibition of DNA repair, DNA methylation). Thus the project will assess cancer risk posed by complex PM mixtures, help to understand host factors that mediate the carcinogenic response of PM and provide an experimental foundation for a targeted hazard/risk assessment.

Two representative publications from supervisors:


5.1 Characterising the aged-senescent endogenous cardiac stem cell population for effective myocardial regeneration and repair

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Project description:
Our findings and those of others show that the adult myocardium harbours a population of resident (endogenous) multi-potent cardiac stem cells (eCSCs). Aging/senescence of the eCSCs determines their function and regenerative capacity. Regulation of this parameter will impact the efficacy of regenerative therapies, considering the majority of patients in need of it are of advanced age. We have found that the number of aged-senescent eCSCs in the human heart increases with age (Figure 1), leading to their impaired proliferation, self-renewal, clonogenicity and cardiomyocyte differentiation.

Moreover, the aged and senescent cells also have a damaging influence over their neighbouring cells by producing deleterious secretions. Potential strategies for mitigating the deleterious effects of senescent cells include eliminating them, interfering with pathways that lead to the senescence-associated growth arrest, and targeting the senescence-associated secretory phenotype (SASP).

In this PhD project you will undertake 3 main objectives:
1. Determine the role of the cardiac SASP on inducing and/or exacerbating eCSC senescence.
2. Elucidate the effect of clearance of senescent eCSCs on growth and differentiation in vitro.
3. Elucidate the effect of clearance of senescent eCSCs on cardiac repair in vivo.

The skill training you will receive will include cell culture, proteomics and mass spectrometry, qRT-PCR, Western blotting, immunostaining, confocal microscopy, and in vivo techniques including the small animal myocardial-infarction regeneration assay.

Two representative publications from supervisors:


Alsharidah M, Lazarus NR, George TE, Agley CC, Velloso CP, Harridge SDR. Primary human muscle precursor cells obtained from young and old donors produce similar proliferative, differentiation and senescent profiles in culture. Aging Cell. 2013, 12(3):333-44
6.1 Unravelling the impact of sequence, expression and genome processing variation in human mitochondria across individuals and tissues.

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Project description:
Mitochondria are involved in a wide range of cellular processes, from energy production to thermogenesis and cell death. Approximately 600 mutations in the mitochondrial genome have been implicated in disease, and mutations in both nuclear and mitochondrial genes are associated with diseases as diverse as Diabetes, Parkinson’s Disease and Cancer. Despite this, differences in the fundamental genetic characteristics of mitochondria across a population and between different tissue types are not fully understood. This project will characterise variation in mitochondrial DNA and RNA across a large number of individuals and tissues within the deeply-phenotyped TwinsUK study. The study will investigate variability in mitochondrial transcription and post-transcriptional processing across the population and in relation to concurrently measured biomedical traits.

The student will be taught how to manipulate high-throughput sequencing data to answer important biological questions. This will include data mapping, normalisation and testing. More broadly the student will undergo training in statistical analysis, bioinformatics (including programming) and scientific writing:

Year 1: Investigate variation in mitochondrial genetic variation across individuals and tissues using RNA sequencing data. Determine whether variation is heritable and/or tissue specific.
Year 2: Investigate variation in the post-transcriptional modification of mitochondrial RNA across individuals and compare how processing varies across tissues and with phenotypes. Determine whether the genetic drivers of such events are tissue specific.
Year 3: Characterization of mitochondrial gene expression: investigating how expression patterns of mitochondrial genes vary across individuals, tissues and phenotypes, as well as identifying the genetic drivers of such variation.
Year 4: Write-up of results.

Two representative publications from supervisors:


7.1 Modulation of myofilament Ca\(^{2+}\) sensitivity in heart muscle cells by positive inotropic agents

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Project description:

One emerging approach to improve contractility in the failing heart is the administration of Ca\(^{2+}\) sensitizers (e.g. levosimendan). While the primary target of these sensitizers is thought to be cardiac troponin, the molecular details are not fully understood. We recently showed that levosimendan and its analog could form a reversible covalent bond with C84 of cardiac troponin C and proposed that the Ca\(^{2+}\)-sensitizing function of levosimendan is to stabilize the Ca\(^{2+}\)-bound conformation of cTnC. To test this hypothesis, we are going to apply the Fluorescence for In Situ Structure (FISS) technique to study both structural and functional effect of levosimendan and its analog in cardiac muscle cells.

Performing FISS experiment requires a unique combination of expertise, ranging from molecular biology, protein biochemistry, muscle physiology to biophysics of data interpretation. Depends on the student’s background and previous experience, he/she will have opportunities to be trained in all these skills.

During the 1st year of the project, the main focus for the student will be the trainings in basic skills required for the FISS experiment, such as molecular biology, protein biochemistry and muscle physiology. In the 2nd year, the student will perform FISS to complete the control experiments and to start experiments with Ca\(^{2+}\) sensitizers. Trainings in biophysics of data interpretation will be involved for analysing the experiment data. In the 3rd year, depending on the type of program the student chooses, the student will complete the experiments and data analysis, and start thesis writing.

Two representative publications from supervisors:


8.1 Determining how protection of ABCE1 contributes to maintenance of tumour growth: a structure and function approach.

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Project description:
The ATP binding cassette enzyme ABCE1 is part of a complex that is essential for ribosome synthesis and function. We have identified two other members of the complex, Lto1 and Yae1, one of which is amplified in tumours. ABCE1 bears two iron-sulphur (Fe-S) clusters, both essential for ABCE1 function. These clusters would be damaged by the elevated levels of pro-oxidants present in tumours. Preliminary data indicates that Lto1 and Yae1 prevent this oxidative damage. The objective of the research project is to understand how Lto1/Yae1 protects the ABCE1 complex. This will be achieved by determining the 3D-structure of the ABCE1/Lto1/Yae1 heterotrimer.

Year 1:
ABCE1 will be cloned into an E.coli expression vector. Following purification, the Fe-S clusters will be reconstituted under anaerobic conditions in the presence of the IscU Fe-S cluster re-assembly enzyme using techniques established in Pastore’s lab. UV absorbance spectra will be used to assess holo-protein stability.

Years 2&3:
Yae1/Lto1 will be expressed in E.coli. Purification of the heterodimer will be followed by assembly of the ABCE1/Yae1/Lto1 complex, and determination of its structure via X-ray crystallography or by a combination of NMR and SAXS studies.

Year 4:
A series of mutants will be expressed in vivo which should compromise the function of the heterotrimer. This will be carried out by exploiting the tractable molecular genetics of the baker’s yeast model system, and will be used to assess the physiological relevance of the structure.
Training: Protein purification/biophysical techniques (Pastore lab). Gene cloning/yeast molecular genetic analysis (Panaretou lab).

Two representative publications from supervisors:

Zhai C, Li Y, Mascarenhas C, Lin Q, Li K, Vyrides I, Grant CM, Panaretou B. (2014) The function of ORAOV1/LTO1, a gene that is overexpressed frequently in cancer: essential roles in the function and biogenesis of the ribosome. Oncogene 33: 484

9.1 Molecular Organisation at the membrane of Antigen Presenting Cells

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Project description:
During an immune response, T cells form a junction with antigen presenting cells (APCs) called an immunological synapse. Antigenic peptide on the surface of APCs is recognised by T Cell Receptors (TCRs) on the surface of T cells. This sets off a chain of events in the T cell including the formation of microclusters of key signalling intermediates such as TCR, Lck and LAT, membrane condensation and actin cytoskeletal rearrangement. Less well understood is the role of protein clustering and the cytoskeleton in the APC and how they regulate this process.

This project will investigate molecular organisation in APCs during synapse formation using the latest advances in fluorescence microscopy and image analysis. The student will extensively use single-molecule super-resolution microscopy (which won the 2014 Nobel Prize for Chemistry) and statistical analysis of protein clustering at the interface. We will also use live-cell super-resolution microscopy to analyse the dynamics of the APC cortical actin cytoskeleton. The imaging will be performed in a number of model systems including artificial planar bilayers mimicking the APC and cell-cell interactions.

Year 1 would be to establish the experimental systems and become familiar with the advanced microscopy and data analysis. Year 2 would acquire experimental data on clustering in artificial bilayers mimicking the APC. Year 3 would examine clustering in cell-cell interactions and year 4 would include live-cell super-resolution imaging. The student would gain skills in advanced fluorescence microscopy, statistics and image analysis, membrane biophysics and immunology.

Two representative publications from supervisors:


10.1 The role of sarcomeric stress in diseased mechanical environments

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Project description:
Inherited cardiomyopathies are frequently caused by mutations to sarcomeric proteins. Their loss of function causes stress on the sarcomere that is signalled to the nucleus to change the cell behaviour. Heart disease and heart failure are also linked to changes in the composition of the extracellular matrix that lead to an increase in rigidity, which further enhances the stress on the sarcomere.

Here we will analyse the role of disease-linked sarcomeric proteins for force production and sarcomeric integrity in changing mechanical environments.

For this we will use existing (MyBP-C3) and also create new knock out human IPS cell lines for sarcomeric proteins (alpha-actinin 2, Myomesin 1) via CRISPR genome-editing. The cells are differentiated into cardiomyocytes and then plated on substrates of varying rigidity. These also include micro patterned adhesive islands that allow attachment of multiple cells (for effects on the intercalated disc) and force-sensing pillar arrays. We will analyse the effect onto the integrity of the sarcomere by confocal and super resolution microscopy and on the force output (shape and magnitude of myofibrillar contraction peaks) with high frequency analysis of the pillar displacements. Changes on gene expression pattern will be analysed by microscopy, western blotting and qPCR.

The objectives for each year include:
1) Differentiation and analysis of MYBP-C3 and control cells. Generating knock out cell lines for alpha-actinin 2 and Myomesin 1
2) Analysis of sarcomere structures and sarcomeric contractions of control and mutant cell lines.
3) Analysis of rigidity effects by confocal microscopy, western blotting and qPCR.

Two representative publications from supervisors:


11.1 Characterisation of the c-Fos mediated immune response against a novel cytolytic peptide toxin

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Project description:  
We have identified the first cytolytic peptide toxin (Candidalysin) in any human fungal pathogen. Candidalysin is exclusively expressed in hyphal filaments of Candida albicans and is essential for mucosal pathogenesis, epithelial activation and damage induction (Nature; In Revision). Central to innate immunity induction by Candidalysin is activation of the transcription factor c-Fos. Currently, the mechanisms regulating Candidalysin gene expression in the fungus and the identity of c-Fos target genes in epithelial cells are unknown. Therefore, the objectives of this proposal are to (i) determine how Candidalysin is regulated and (ii) determine which epithelial cell responses are driven by Candidalysin through c-Fos activity.

To determine how Candidalysin is regulated, we will clone the upstream flanking DNA region of the Candidalysin gene to create reporter gene constructs. Introduction of deletion and substitution mutations will identify the key regulatory regions and elements for Candidalysin gene expression. Next, we will use Next Generation Sequencing technologies to perform ChIP-Seq to determine which genomic (gene regulatory) regions c-Fos associates with in response to Candidalysin. By comparing these data with the Candidalysin-induced epithelial transcriptome (RNA-Seq), we will determine the target genes and pathways regulated by c-Fos. Finally, the importance of Candidalysin gene regulation and c-Fos target genes in host immune responses to C. albicans infection will be determined in vitro and in vivo.

These findings will identify key events in Candidalysin regulation and protective host responses that can be targeted for novel antifungal therapies and will define new mechanisms in mucosal-microbial responses.

Two representative publications from supervisors:


12.1 Regulation of human immunodeficiency virus type 1 (HIV-1) gene expression by SR proteins

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Project description:  
Cellular RNA binding proteins are essential for HIV-1 replication. Characterising how the virus uses these proteins to replicate is crucial to understand the viral life cycle and develop new antiviral treatments. We have identified specific members of the SR family of cellular RNA binding proteins that regulate HIV-1 gene expression and virus production. However, we do not yet understand how they control the different steps of HIV-1 gene expression. Importantly, SR proteins are potential anti-HIV drug targets and further understanding of their mechanism(s) of action will help elucidate how they regulate HIV-1 replication.

To understand how SR proteins control HIV-1 gene expression, it is essential to determine where they bind HIV-1 RNA at single nucleotide resolution and to determine the binding sites that are functionally important. To identify and characterise the in vivo binding sites for SR proteins on the HIV-1 genomic RNA, the student will:

1. Use individual-nucleotide resolution cross-linking and immunoprecipitation (iCLIP) to identify the binding sites in the HIV-1 genome for wild type SR proteins and loss-of-function mutant proteins with changes in their RNA binding domains.

2. Compare the binding sites in the HIV-1 genome between the different SR proteins and between the wild type versus mutant proteins.

3. Mutate SR protein binding sites in the HIV-1 genome and determine their importance for viral replication.

This project is ideal for a student with a strong interest in performing both wet bench experiments and the bioinformatic analysis of the data they generate.

Two representative publications from supervisors:


13.1 Engineering the IgE receptor CD23 to examine the role of calcium

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Project description:
The Immunoglobulin E receptor CD23/FcεRII is a pleiotropic cell surface receptor found on B cells, where its roles include regulation of IgE synthesis and enhanced antigen presentation, which leads to epitope spreading. CD23 is a C-type Lectin, and other members of the superfamily bind two calcium ions, one of which helps bind carbohydrates. Human CD23 (but not rodent) cannot bind the calcium ion involved in carbohydrate interactions and has no clear need for carbohydrate in binding to IgE. Our crystal structures1+2 of CD23 (± calcium and IgE) imply that the modified calcium ion-binding site may enhance IgE binding.

We propose to test this hypothesis by restoring the missing calcium binding site in human CD23, removing it from mouse CD23 and examining the biological effects. This will help us to understand the inter-species differences in IgE biology and assist development of CD23/IgE blocking molecules for the treatment of allergy.

Aims: Y1) You will engineer the calcium and IgE binding sites of human and mouse CD23 to generate a panel with different calcium binding stoichiometries, and variants that influence the CD23 binding interface for IgE. Y2) You will assay the effect of these mutations and solve the crystal structures of the mutant proteins. Y3/4) You will create mutant mammalian cell-lines and examine the biological effect of the mutants on protein sorting in endosomes.

Skills: Protein engineering; BIAcore kinetic protein binding assays and ITC for calcium binding; Molecular Dynamics simulations and structure determination by X-ray crystallography; gene editing to produce mutant cell-lines.

Two representative publications from supervisors:


14.1 Exploring the general mechanisms of cancer cell migration using live cell imaging combined with computer vision techniques and computational modelling.

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Project description:  
Cancer is a devastating disease, with more than one in three people in the UK developing cancer in their lifetime (2011, CR-UK). Cancer metastasis is the most frequent cause of cancer-associated mortality. The acquisition of migratory abilities of cancer cells is an important prerequisite for their metastatic spread throughout the body. Yet, surprisingly little is known about the exact fundamental differences in migratory mechanisms of normal versus cancer cells. We will exploit our ability to image the dynamics of the intracellular migratory machinery of cells at unprecedented spatio-temporal resolution to elucidate the mechanisms underlying the increased migratory potential of cancer cells.

In the proposed project you will explore the general mechanisms of cancer cell migration using live-cell imaging combined with computer vision techniques and computational modelling. In the first year of the project you will learn cloning, cell culture and live-cell imaging techniques to compare the migratory behaviour of normal, non-transformed cells with that of cancer cells of increasing metastatic abilities. You will also acquire knowledge of techniques developed in the lab to image the migratory machinery of these cells and develop analytical approaches to quantify the interplay of these intracellular dynamics with gross cellular movement. In the following years you will use computer vision techniques and computational modelling to analyse the different migratory abilities of cancer and normal cells in detail. The CRISPR-CAS mediated knockout or overexpression of key controllers of the actin migratory machinery will allow you to interrogate key differences in cancer versus normal cell migratory behaviour.

Two representative publications from supervisors:


15.1 Defining the factors that confer limb-forming potential to progenitor cells

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Project description:
The broad aims of the project are to increase knowledge of the factors that direct limb formation to increase understanding of the aetiology and pathogenesis of congenital limb disorders and to develop ways to harness this knowledge in regenerative strategies.

The project will start using existing transgenic reporter lines and primary culture methods that will be refined and developed over the course of the project. One important goal will be test and establish the utility of primary or stable cell lines produced as an assay tool for teratogens.

Year 1 milestone - Identify and characterise the combination of factors that are required to transform a mesoderm cell into a limb progenitor. Using chick and mouse reporter transgenics as a source, cells from the limb bud and embryo flank will be exposed to factors in primary culture. Complementary in ovo approaches will also be used in the chick (ref 1).

Year 2 milestone - Establish culture conditions to maintain and expand limb progenitors and to push these progenitors toward specific cell fates eg. Chondrogenesis and tenogenesis

Year 3 milestone - Use iPSC techniques to direct cells to a limb progenitor fate.

The student will be trained in the embryology and use of animal model systems (chick and mouse), genetics and genetic manipulation of the mouse, in addition to learning and developing cell culture in vitro assays and iPSC technology. The results of this project will contribute to regenerative strategies, tissue engineering and stem cell technologies.

Two representative publications from supervisors:

RA acts in a coherent feed-forward mechanism with Tbx5 to control limb bud induction and initiation
Cell Reports 12(5):879-91

A combination of activation and repression by a collinear Hox code controls forelimb-restricted expression of Tbx5 and reveals Hox protein specificity.
PLOS Genetics 10(3)
16.1 Understanding Aurora kinase functions at the nuclear periphery

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Project description:
Eukaryotic genome is compartmentalized inside the nucleus delimited by the double-membrane nuclear envelope (NE). The NE must be dynamically restructured during mitosis to allow chromosome segregation and formation of the daughter nuclei. Many cell types including human cells and the unusual model yeast S. japonicus break the NE for the duration of mitosis.

We have recently made an important discovery that Aurora kinase promotes NE breakdown and reassembly in S. japonicus. Alterations in Aurora signalling have been long associated with errors in chromosome segregation and linked to aneuploidy in human cancers but most research has been concentrated on Aurora functions at the chromosomes and the mitotic spindle rather than the nuclear envelope. We use a genetically tractable organism S. japonicus as a discovery tool to pinpoint the mechanism for Aurora function in mitotic NE dynamics. We will eventually translate our research to human cells with a view of understanding how Aurora contributes to proper execution of mitosis at the nuclear periphery and how its malfunction may lead to disease.

Year 1. Development of genetically encoded FRET-based biosensors to report Aurora activity at the nuclear periphery and NE kinase tethers (SO and SAB).

Year 2. Understanding spatiotemporal regulation of Aurora signalling at the NE using FRET and other techniques (SO and SAB).

Year 3. Identification of Aurora NE targets and their functional validation in S. japonicus (SO).

Year 4. Based on the previous experimental results, we will study the mechanistic details of Aurora function at the mammalian NE (SO and SAB).

Two representative publications from supervisors:


18.1 Defining the interaction between HLA-DR3 and regulatory T cells in a murine model of autoimmune hepatitis

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Co-Supervisor 2: Dr Harry Antoniades, Senior Lecturer at both KCL and Imperial College London
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Project description:
Autoimmune hepatitis (AIH) is a severe liver disorder. Loss of tolerance exerted by immunoregulatory CD4^{high}CD25^{high} FOXP3^{high} T cells (Tregs) and MER tyrosine kinase (MERTK) macrophages results in persistent liver damage. Despite lifelong immunosuppression, 45% of AIH patients will progress to cirrhosis. AIH is associated with human leukocyte antigen (HLA) class II DRB1*0301 (HLA-DR3) with HLA-DR3^{apos} patients exacerbate liver damage. In our newly established mouse model for AIH, we demonstrate that mice bearing human HLA-DR3 (Yuksel M, et al. 2015) behave similarly to HLA-DR3^{apos} patients. In addition, there is a link between the suppressive function of Tregs and the possession of HLA-DR3 in mice and humans. Our group has also identified a novel regulator of pro-resorptive/tolerogenic macrophages, MER-tyrosine kinase, in liver inflammatory pathologies and have shown a deficiency of these antigen-presenting cells in autoimmune-like hepatitis, the latter characterised by excessive pro-inflammatory responses (Bernsmeier C, et al. 2015).

We hypothesize that Tregs, experiencing less frequent TCR signalling by inefficient antigen presenting capacity of HLA-DR3^{apos} antigen-presenting cells, become dysfunctional and anergic or apoptotic. TCR signalling elicits intracellular calcium signalling is vital for Treg suppressive function. In vivo imaging of lymphoid tissue by two-photon microscopy (TPM) will allow us to assess the signalling in immune cells.

We will define whether: 1) HLA-DR3 has an altered TCR interaction influencing the generation of autoantigen specific Tregs; 2) MERTK expression on monocytes/DC/macrophages in different tissues influences Treg function; 3) immunisation with low dose IL-2/anti-IL-2 complex could re-establish tolerance.

Two representative publications from supervisors:


Analysis by genome editing and live imaging of novel host factors required for plasma membrane repair and HIV-1 and Ebola virus budding.

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Project description:
The Endosomal Sorting Complex Required for Transport (ESCRT) pathway mediates scission of thin membranous tethers found in the final steps of several fundamental processes, including multivesicular body (MVB) formation, cytokinetic abscission and nuclear envelope reformation. Because ESCRT subunits are the only cellular machinery capable of inducing membrane invaginations that protrude away from the cytoplasm, enveloped viruses, such as HIV-1 and Ebola, hijack the ESCRTs to facilitate their budding. Similarly, the ESCRT machinery plays an important role in plasma membrane repair upon injury by pore-forming bacterial toxins by facilitating shedding of the damaged part of the membrane. Thus, understanding ESCRT function is of considerable importance both in health and disease.

ESCRT-mediated membrane remodelling and scission needs to be coordinated with the accompanying rearrangements of the underlying cytoskeleton. However, how the ESCRT machinery relates to the cytoskeletal components during normal and pathological conditions remains poorly understood. Recent data obtained in the laboratory from a proteomic screen of purified ESCRT-containing complexes has identified a network of proteins important for the regulation of both actin and membrane dynamics.

This project will use a multidisciplinary approach involving the latest biochemical, genetic and microscopy techniques to analyse the function of these novel ESCRT-binding proteins in diverse processes, including membrane repair and viral budding. Briefly, the student will use the RNA-guided CRISPR/Cas9 nuclease system for genome editing to specifically deplete, introduce point mutations or create reporter constructs of the newly identified ESCRT-associated proteins and will employ real-time fluorescent and super-resolution microscopy to determine their role during normal and pathogenic conditions.

Two representative publications from supervisors:

Agromayor M and Martin-Serrano J. Knowing when to cut and run: mechanisms that control cytokinetic abscission. Trends Cell Biol. 2013 Sep;23(9):433-41

20.1 Understanding conformational dynamics and allosteric modulation in immunoglobulin E

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Project description:
Immunoglobulin E (IgE) is the central effector of allergic responses. Its effects are mediated through its cellular receptors, FcεRI and CD23, which interact with the Fc region of IgE (IgE-Fc). IgE-mediated crosslinking of FcεRI is the cause of the immediate hypersensitivity reaction that results in either systemic anaphylaxis or tissue-specific reactions in the airways (asthma), nasal mucosa (rhinitis, hayfever), gut (food allergy) and skin (atopic dermatitis).

Using X-ray crystallography and NMR spectroscopy, we have defined three-dimensional structures and interactions for a variety of IgE-Fc structures and complexes, and identified an allosteric communication pathway that prevents simultaneous engagement of CD23 and FcεRI receptors. Our initial analyses of the allosteric processes occurring in IgE suggest the use of at least three distinct mechanisms: (i) what could be thought of as “traditional” allostery, long-range rigid body motions from domain reorientations, (ii) smaller scale, intradomain motions that make use of IgE’s unusual structural plasticity, and (iii) dynamically-driven allosteric effects, which result from changes in dynamics (and hence entropic energy changes). We propose to use a broad-based structural and biophysical approach to elucidate these mechanisms of allosteric regulation within the IgE molecule. We anticipate that these studies will define new opportunities for controlling IgE-mediated responses, and offer the possibility for novel anti-IgE therapies with new functionalities and greater effectiveness.

This project offers training in structural (e.g. x-ray crystallography and NMR), biophysical (e.g. native mass spectrometry, stopped-flow kinetic methods, and calorimetry) and computational studies (e.g. molecular dynamics simulations, and advanced modelling using hybrid approaches).

Two representative publications from supervisors:


22.1 Mechanotransduction in Cardiac Myocytes

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Project description:
The structure and function of the heart centres around force, and force influences the structure and development of cardiac myocytes. In this project, you will investigate the role of mechanical stress in remodelling of the cardiac sarcomere. The mechanically regulated dynamics and protein turnover via autophagy and proteosomal degradation can be perturbed by mutations in Z-disk and M-band associated mechanosensor complexes, which can ultimately lead to cardiomyopathy and heart failure. You will investigate how dynamics of Z-disk proteins affect the mechanical properties of cardiac myocytes. This will involve labelling alpha-actinin (the Z-disk structural actin-crosslinker), BAG3 (an autophagy adaptor and major myopathy target) and myotilin (an alpha-actinin binding protein shuttling between Z-disks and M-bands and a major myopathy target) and imaging the cells while applying local mechanical perturbations. This will reveal how structures such as Z-disks change and respond to the local mechanical properties of the cell. You will also use iPSC-derived cardiomyocytes from controls and cardiomyopathy samples to understand how human mutations impact on these responses.

Year 1
Develop contractility assay for fluorescence videos of cardiac myocyte.

Year 2
Apply contractility assay to investigate the behaviour of Z-disk and associated mutations.
Develop feedback system to allow AFM probing of cells.

Year 3/4
Apply AFM and superresolution imaging to iPSC-derived cells with mutations in Z-disk and associated proteins.

Skills training: Cardiomyocyte cell culture, cell transfection and use of adenoviral vectors, protein biochemistry, live-cell imaging, super-resolution fluorescence microscopy, atomic force microscopy, programming, data analysis, computer vision.

Two representative publications from supervisors:

23.1 Molecular regulation of collective cell migration.

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Project description:
Cell migration is fundamental for life, from embryo formation to wound healing. The tight control of movement initiation, pathway recognition and movement arrest requires complex molecular and cellular interactions. Moreover, the deregulation of any of these steps leads to pathologies as cancer metastasis. This project will use zebrafish neural crest cells (NCs) as a model system to study these interactions. We have shown that trunk NCs (TNCs) undergo collective migration and are directed by a single leader cell. How this leader cell find its pathway remains unknown. TNCs and motor neuron axons (MNA) extend ventrally sharing the same route. Moreover, classic and molecular experiments shown that alterations to the substrate concomitantly affect MNA and TNCs migration, suggesting their movement may be interdependent. Our preliminary data indicates that extending MNA instruct leaders directionality through the neuregulin/ErbB pathway. The first aim of this project will be to characterize TNCs-MNA relationship and to determine the role of MNA extension in TNCs migration. This will be attained using quantitative live imaging and single cell ablation experiments. Next, we will define the role of MNA neureguin in TNCs migration, by in vivo imaging TNCs migration in neuregulin mutants. Finally we will study the localization of ErbB signaling using a FRET in vivo sensor and the role of this receptor signaling in TNCs migration by in vivo this process under inducible gain- and loss-of-function conditions. In conclusion, using a combination of live imaging, molecular and genetic tools this project will unveil the molecular and cellular interactions by which collectively migrating neural crest define their pathway.

Two representative publications from supervisors:


24.1 Mechanism of Action of oncogenic microRNAs in the Central Nervous System

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Project description:
Brain tumours are the major cause of cancer-related deaths in people under 40. Understanding their variety in terms of genetics, epigenetics, cell of origin and phenotypic properties is critical for developing much needed personalised therapies.

We have identified a novel neural tumour type in which ectopically dividing cells co-express markers of neural stem cells and of differentiated cells. Cell fate ambiguity has been described in human leukaemias raising the possibility of a general oncogenic mechanism. We wish to understand how “fate-ambivalent” cells originate in a Drosophila neural tumour model, and whether the principle and molecular mechanism(s) apply to human cancers.

MicroRNAs are small non-coding RNAs that regulate gene expression by modulating translation and stability of their mRNA targets. Many microRNAs are deregulated in cancer and can act as tumour suppressors or oncogenes (onco-microRNAs). Overexpression of several evolutionarily conserved microRNAs in Drosophila neural lineages leads to fate-ambivalent tumourigenic cells (our ongoing work). Of these, microRNA-9 and microRNA-34 have been implicated in human glioma. The student will:

i. Further characterise onco-microRNAs functionally and immunohistochemically (eg. time and cell type of action). (Rotation + Year 1)

ii. Determine onco-microRNA targets, combining: TU–tagging followed by RNAseq; biochemistry (isolation of Argonaute-associated RNAs); and target-prediction algorithms. (Years 2+3)

iii. Investigate conserved onco-microRNAs and their target pathways in mammalian neural differentiation and proliferation models (Years 3+4)

Learning opportunities: brain development and anatomy; stem cells, differentiation, cell fates and cancer; classical and molecular genetics; tissue culture and cell biology; imaging; molecular biology and biochemistry; bioinformatics and network analyses.

Two representative publications from supervisors:


25.1 Role of TFR modulation on antimicrobial resistance and infections caused by Gram-negative bacteria.

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Project description:
Multidrug-resistant (MDR) pathogens have emerged as a major concern for public health and there are dwindling treatment options for MDR Gram-negative pathogens. The TetR family of regulators (TFRs) are widely associated with antimicrobial resistance (AMR) and the regulation MDR efflux-pumps. The proposed project will use a novel series of sequence selective molecules that can modulate the expression of TFRs and study their role in antimicrobial resistance in Gram-negative bacteria and infectious disease caused by Gram-negative bacteria in animal models. An ongoing collaboration between Kings College London and Public Health England has identified a new class of sequence selective compound that bind to the promoter region of the Tet-repressor binding site and modulate the function of TFRs. The student will explore the phenomenon, and the finding from the study could help the researchers to understand the role TFRs in conferring multi-drug resistance and target this to develop broad-spectrum antibiotics or reviving antibiotics that have become ineffective against resistant bacteria.

Year 1: Initial synthesis of small-focused libraries around the identified lead structures and early microbiological validation of the chemical series at Public Health England.

Year 2: Use the synthesised compounds to use them as chemical tools to study the role of substitutions on modulation of TFRs.

Year 3 & 4:
i) Determine the effects of tool compounds on TFR modulation and link it with antimicrobial resistance.
ii) Generate Gram-negative bacterial cell lines with Tet-R mutations to understand the role of TFR modulation on Gram-negative infection in animal models.

Two representative publications from supervisors:


26.1 A cellular approach to understand congenital eye malformations

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Project description:
Anophthalmia and microphthalmia (AM) are among the leading causes of congenital blindness accounting for 3-11% of childhood blindness. While recent studies have identified some underlying mutations in patients and animal models, the aetiology is poorly understood: even when the gene is known, the cell behaviours causing eye defects are not. This project aims to close this gap by investigating the cellular mechanisms that initiate eye formation in normal and mutant animal as models for congenital AM.

We have shown that optic vesicle outgrowth initiates from a region in the neural tube, the posterior optic vesicle boundary (pOVB), where cells stop proliferating and undergo basal constriction. The mechanisms that drive basal constriction are not well understood; our studies implicate focal adhesion kinase and integrins as key components. The proposed Ph.D. project will expand on these studies. State-of-the-art multiphoton live imaging in chick embryos and mouse organoid cultures will be used to examine the cell dynamics at high resolution and test the effects of alterations in signalling pathways that we have found to converge at the pOVB, including Notch and PCP, as well as cytoskeletal and adhesion molecules. Phenotype-genotype links will thus be revealed at the mechanistic cellular level.

Understanding eye morphogenesis will be useful for developing new diagnostic criteria for AM and interpreting new candidate genes identified in patients by a collaborator, Prof. David Fitzpatrick (Edinburgh). It will also provide new insight into a basic cellular mechanism, basal constriction, which is used throughout development and repair to shape organs and tissues.

Two representative publications from supervisors:


27.1 Using human iPS cells to identify genetic variants that influence cellular differentiation

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Project description:  
The project will contribute to the Wellcome Trust/MRC-funded Human Pluripotent Stem Cell Initiative (www.hipsci.org), which is using human iPS cells from healthy individuals to understand the genetic basis of inter-individual variability of cellular functions. By applying cell-based assays to distinguish causative from correlative genetic variants in healthy and diseased individuals we aim to develop an in vitro approach to precision medicine. In the first phase of the project we have analysed human iPS cells in the undifferentiated, pluripotent state. The PhD student who joins the project will contribute to analysing differentiation into the three embryonic germ layers, using a technique that is based on that of Warmflash et al. (Nat Methods 2014, 11:847-854). Supervisor 1 has extensive experience of high throughput assays of cell behaviour and using computational tools to integrate genomic, proteomic and cell phenotypic data. Supervisor 1 has considerable experience of inducing pluripotent stem cells to differentiate.

Year 1: learn how to culture human iPS cells; optimise the differentiation assays and develop analysis pipeline using high content imaging data
Year 2: screen iPS cells from healthy individuals and identify genetic variants for further analysis
Years 3 and 4: explore how the selected variants impact cell behaviour, for example using genome-editing tools.

Skills training: culturing human iPS cells; creating micro-patterned surfaces to induce differentiation; integrating different types of datasets; Crispr/Cas9 technology; opportunities for collaborative working with Hipsci partners.

Two representative publications from supervisors:

28.1 Mef2 role in control of striated muscle growth and regeneration in heart and skeletal muscle

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Project description:  
What controls the final size of an organ or tissue such as muscle or heart and how it is regulated in regeneration following wounding or disease is essential biological questions. In this project we aim to use to try and answer these questions.

The Mef2 family of transcription factors couples various developmental, physiological and pathological signals to cell-specific transcription in many cell types. It is highly expressed in muscle, heart and brain tissue and was implicated in various genetic disorders. We have shown previously that a combination of Mef2 proteins is necessary for normal differentiation in the embryo of both skeletal muscle fibres and cardiomyocytes in the heart. Mef2s are also thought to play an important role in repair and regeneration of muscle and heart following disease or injury.

The levels of Mef2 have a strong implications on the number of differentiated cells. For example, Mef2c mutant fish fail to generate normal heart muscle (ref2) and have fewer cardiomyocytes. Nevertheless, mutants recover, possibly through adaptive cardiomyocyte hypertrophy. The project will investigate the roles of Mef2 and other myogenic genes in homeostatic control of heart and skeletal muscle growth, and the regulation of stem cell number.

We will use transgenic zebrafish with fluorescently labelled muscle or heart cells, combined with the creation of novel genetic mutants that can be activated in a time and tissue-specific manner (generated by CRISPR/TALEN genome editing) to study these questions.

Two representative publications from supervisors:


29.1 Ebola virus disease: antibody discovery and biomarkers of survival.

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Supervisor 2: Prof Franca Fraternali
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Project description:
2014/15 saw an unprecedented epidemic of Ebola virus disease in West Africa, with nearly 30,000 infected and over 11,000 have died as a result. The worst is over but there are still occasional new cases, and the reactivation of virus in immune privileged sites may be a problem. Kings is in a unique position to help with the healthcare effort as well as with understanding the mechanisms of infectivity of the virus and the host response to the virus. Deborah Dunn-Walters has been working with Kings Sierra Leone partnership in Freetown, and other UK collaborators, to organise collection of safe samples from Ebola patients for further study. Together with other colleagues in DIIID we are building a program of research in Ebola virus disease.

This particular project will focus on the B cell responses to the disease. In particular in using our molecular methods to identify which antibodies may be associated with survival from the disease so that they can be used in therapeutic and diagnostic approaches to combat the disease.

We will screen Ebola survivor plasma samples to identify those with high titres of neutralising antibodies. RNA will be prepared from RNA from matched blood samples and the Ig genes amplified to incorporate multiplex identifiers and produce sufficient material for high throughput sequencing. The sequence data will be analysed to produce a table of Ig gene features linked with metadata, which will be interrogated to determine: a) general repertoire characteristics in survivors versus controls versus non-survivors. b) Ig gene features common to multiple survivor samples. Ig gene types that are found to be expressed in multiple survivor samples, or expanded in individual samples, will be cloned and expressed. These antibodies will be screened for Ebola neutralising activity using a safe, class II containment, assay system. This is a safe way to discover new anti-Ebola antibodies for therapeutic use. Experiments to look for genetic markers associated with survival can also be conducted.

The Dunn-Walters lab has extensive expertise in immunoglobulin gene isolation, high throughput sequencing and antibody cloning/expression.

The Fraternali lab has extensive expertise in bioinformatics, molecular modelling, protein interaction and systems biology. Both supervisors currently work together on the flagship MRC/BBSRC programmes MABRA – combining wet lab work with in silico analysis of samples data and modelling of antibodies from patients blood.

Two representative publications from supervisors:

YC Wu, D Kipling, HS Leong, V Martin, AA Ademokun, DK Dunn-Walters High-throughput immunoglobulin repertoire analysis distinguishes between human IgM memory and switched memory B-cell populations Blood 116 (7), 1070-1078

Chung SS, Pandini A, Annibale A, Coolen AC, Thomas NS, Fraternali F.
Identification of therapeutic targets for the treatment of severe skin autoinflammation

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Project description:
Abnormal innate immune activation (autoinflammation) underlies a range of skin disorders that have profound effects on quality of life. The focus of this project is generalised pustular psoriasis (GPP), a potentially life-threatening condition, which will be investigated as a model of skin autoinflammation. Our goal is to characterise novel GPP genes, in order to illuminate innate immune pathogenic pathways, which will also be relevant to common skin diseases such as severe acne.

During the rotation project (or the first year of a 4-year programme), the student will analyse a GPP whole-exome sequence dataset. He/she will prioritise candidate genes for follow-up, based on their expression pattern and their overlap with loci identified in genome-wide association studies of severe acne.

In subsequent years, the student will:
- Investigate the impact of GPP mutations in-vitro, through the characterization of knock-out or knock-in cell lines, generated by CRISPR-Cas9 genome editing
- Characterise the effect of acne susceptibility alleles, using the experimental systems developed in the earlier phase of the project
- Determine whether the impact of genetic defects can be reversed ex-vivo, by treating patient keratinocytes with pharmacological modulators of disease associated pathways

These experiments are expected to improve our understanding of skin autoinflammation, paving the way for the development of targeted therapeutics.

The work will be undertaken in a multidisciplinary environment, underpinned by a longstanding collaboration between geneticists and dermatologists. This set up equips students with a broad range of laboratory and analytical skills, enabling them to publish their findings in leading genetics journals.

Two representative publications from supervisors:


K31.1 Developmental basis of skin diversity

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Co-Supervisor 2: Prof Anthony Graham  
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Name of Collaborating Clinician (if not one of the two co-supervisors) Dr Alastair Mackenzie Ross  
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Project description:
The skin exhibits anatomical diversity, with different regions fulfilling different functions; the skin over our heads is covered in hair, the skin of our hands and feet is quite different that of our abdomen. Regional differences in the skin emerge during development and these are imparted by the dermis, which has a complex developmental history. Specifically, the dermis of the back is derived from somites, derivatives of the paraxial mesoderm, the dermis of the limbs is derived from lateral plate mesoderm and the dermis of the face from neural crest cells. We hypothesize that the positionally distinct dermal features (including repair and regeneration potential, and susceptibility to site-specific skin diseases) reflect the developmental origin of the tissue.

The objectives of this project are to:
1) Determine if the adult dermis from different regions of our bodies retains a gene expression signature reflecting their developmental origins and if they exist, define their distinct gene regulatory networks.
2) Determine if dermis of different embryonic origins has different properties during wound healing.

Understanding regional differences in dermis gene regulatory networks (in homeostasis and during wound repair) has important ramifications for how we understand the pathologies that affect different areas of the skin (for example, keloid scars that generally affect the head and neck region).
This project will use a combination of human samples and in vivo models; the student will be trained in many cellular and molecular biology techniques, including primary tissue culture, gene expression profiling (e.g. RNA-seq), western blotting, immunohistochemistry, and microscopy.

Two representative publications from supervisors:


K32.1 Broadly neutralizing antibodies responses against HIV

Co-Supervisor 1: Dr Katie Doores
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Project description:
Approximately 10-30% of HIV infected individuals generate antibodies that are capable of neutralizing a broad range of HIV isolates and these antibodies have been shown to protect against SHIV challenge in Macaque models. Isolation and characterisation of these antibodies has revealed regions of the HIV envelope glycoprotein, gp120/gp41, that are susceptible to antibody binding and re-eliciting these antibodies may be a key step for a successful HIV vaccine. Gp120 is heavily glycosylated with host-derived N-linked glycans and it was previously thought that these glycans shield conserved protein regions from the immune system. However, we have recently shown that a number of the HIV broadly neutralizing antibodies (bnAbs) bind directly to these glycans highlighting them as potential targets for HIV vaccine design. Using unique longitudinal samples from acutely HIV infected patients in the SPARTAC study (N Engl J Med 2013;368:207-17) we will investigate the development of glycan-binding bnAbs in vivo using in vitro neutralization assays, antigen-specific B cell sorting and single genome amplification. We will determine how the evolving glycan shield impacts and directs bnAb development. Ultimately these studies will be used to design immunogens and immunization strategies aimed at re-eliciting these bnAbs through vaccination.

Two representative publications from supervisors:


Investigations of novel redox-dependent cellular stress response mechanisms

**Co-Supervisor 1:** Prof Maria R (Sasi) Conte  
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**Co-Supervisor 2:** Dr Alison Brewer  
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**Name of Collaborating Clinician (if not one of the two co-supervisors):** Dr Ajay Shah  
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**Nrf2-mediated stress-response, Nox4, Redox-signalling, La protein, IRES-dependent translation**

**Project description:**
All cells are at risk of acute and chronic injury provoked by environmental stresses. Nrf2 has emerged as a master transcriptional regulator of a battery of genes involved in cellular defence against oxidative and toxic insult, and misregulation of this protective pathway associates with the progression of many pathologies including neurodegenerative diseases, inflammatory disorders and cancer.

We recently demonstrated that the Nrf2-regulated pathway is activated by Nox4, a key enzyme that generates physiological levels of reactive oxygen species (ROS) which are important modulators of redox-signalling1. The molecular mechanisms which underlie this redox-dependent activation however are not understood. Nrf2 expression is known to be subject to post-transcriptional regulation. Thus Nrf2 mRNA contains an Internal Ribosome Entry Site (IRES) which allows increased protein production in cells under stressed conditions when normal translation initiation is switched off. Intriguingly, this IRES-dependent translation of Nrf2 appears to be redox-sensitive2 and to require the involvement of an RNA binding-protein, the La autoantigen.

The aims of this project are:

i) To ascertain whether Nox4 regulates the redox-modulated translation of Nrf2.

ii) To further determine whether this Nox4-dependent mechanism involves activation and binding of the La protein to the IRES.

iii) To elucidate the functional domains of La necessary for this binding.

iv) To investigate the redox-dependent molecular basis of the activation of La by Nox4.

**Execution of the project will entail a wide range of molecular and cellular biology in addition to biophysical techniques.**

This project will combine the expertise of Dr Alison Brewer on redox-regulation of transcription by ROS, with that of Prof. Sasi Conte on structure-function of the La protein and regulation of gene expression at post-transcriptional level.

**Two representative publications from supervisors:**


K34.1 Generation of stem cell derived 3-D mini liver through understanding of the transcriptional regulation during human hepatic progenitor maturation

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Project description:
Liver failure is an increasing clinical burden claiming over 10,000 lives per year in the UK alone. The only cure for this condition, liver transplantation, is severely limited by donor availability. Tissues derived from induced pluripotent stem cells (iPSCs) provide an effective solution to the problem but are unsuitable for clinical application due to their poor in vivo functionality. We have recently established a culture system capable of maturing primary hepatic progenitors into a 3-Dimensional ‘mini-liver’ with adult phenotype (Figure 1). The aim of this project will be to understand the networks of gene regulation controlling organ formation in this model and apply that new knowledge to advance the quality of our iPSC derived liver tissue as we move it towards clinical trial.

Objective 1 (1st year) Compare the transcriptome (RNA-seq) and epigenome (bisulfite-seq, ATAC-seq, histone ChIP-seq) of mature vs progenitor primary tissue to uncover differentially expressed and/or marked genes that may be responsible for the maturation process.

Objective 2 (2nd year). Apply the findings from Objective 1 and apply into human iPSCs through the use of gene over-expressing or silencing techniques.

Objective 3 (3rd year). Validate efficacy of advanced iPSC hepatic tissue in pre-clinical animal models of liver failure (such as TK-NOG mouse).

The ultimate goal of this project is to understand how liver organogenesis is regulated, with the aim of generating a stem cell based product ready for clinical work up by the end of the project.

Two representative publications from supervisors:


K35.1 External ear defects in mice and men

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Co-Supervisor 2: Prof Dan Jiang PhD FRCSI(Otol) FRCS(ORL-HNS)
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Project description:
Defects in the external ear can affect the ear canal, or the auricle, or both. The ear canal can be missing (atresia) or narrow (stenosis). In cases of atresia (1 in 10,000-15,000 births) canal reconstruction with recreation of the ear canal lining is sometimes attempted (atresiaplasty), or the external ear is bypassed by a bone-anchored hearing aid. We are interested in how the ear canal forms during normal development and what goes wrong in cases of atresia. We aim to study the development of the external ear taking advantage of mouse mutants with external ear defects. In addition we aim to study external ear defects in patients from the Ear Clinic at St Thomas’ Hospital. The project is a collaboration between an expert in ear development in mice (Prof Tucker) and a clinician specialising in ear surgery (Prof Jiang).

Aim 1: To investigate the normal process of external ear formation during mouse embryonic development.
Aim 2: To understand the mechanisms behind ear defects using mouse models of human syndromes associated with external ear defects. These will include 22q11.2 deletion syndrome (Tbx1 mice), Branchio-oto-renal syndrome (Eya1 mice), LADD syndrome (Fgf10 mice), and holoprosencephaly (Gas1 mice).
Aim 3: To analyse CT scans from patients with external ear defects to correlate the findings from the mouse in humans.
Skills training: The student will be trained in a range of molecular biology techniques, anatomy and regenerative biology, while having access to clinical data. In addition critical thinking, presentation and writing skills will be taught.

Two representative publications from supervisors:


K36.1 iPS modelling of Hajdu-Cheney syndrome for therapeutic screening

Co-Supervisor 1: Professor Paul Sharpe
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Project description:
Hajdu-Cheney syndrome is a rare disorder that predominantly affects bone and commonly manifests as progressive loss of bone both generalised (osteoporosis) and localised (acro-osteolysis) from the hands and feet. The disorder is caused by mutations in the NOTCH2 gene. The progressive bone loss phenotype and its early onset lends itself to clinical intervention and obvious targets are therapeutics that modulate the Notch signalling pathway. The bone loss in Hajdu-Cheney patients is caused by increased osteoclast numbers, a terminally differentiated cell type that can only be expanded in vitro from precursors isolated from the spleen and bone marrow. In order to have a robust assay to screen molecules for potential therapeutic effects, osteoclasts carrying specific Hajdu-Chenney NOTCH2 mutations need to maintained in vitro. The only realistic way of achieving this is to generate iPS from patients and differentiate these into skeletal cells.

This project will involve generation of several iPS cell lines from Hajdu-Cheney patients using HIPSCI and differentiation into osteoclasts using protocols established within the department by Professor Agi Grigoriadis. The effects of small molecule modulators of Notch signalling on osteoclast differentiation, size and activity will be assayed.

Recently a mouse model of Hadju-Cheney has been generated (unpublished) and this will be used as an in vivo model to test the effectiveness of molecules showing activity in the in vitro screens. The ultimate objective is to identify molecules and conditions that can reverse progression of the disease and can be tested in patients.

Two representative publications from supervisors:


Simpson et al. (2011) Mutations in NOTCH2 cause Hajdu-Cheney syndrome, a disorder of severe and progressive bone loss. Nature Genetics 43, 303-305
K37.1 The role of mitochondrial DNA as a mediator of chronic inflammation in diabetic nephropathy

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Project description:
Diabetic nephropathy (DN) is a kidney disease which affects >100 million people worldwide and is a leading cause of renal failure and mortality. Data from our group has linked mitochondrial dysfunction and MtDNA changes with DN. We have shown, using animal/cell models, and patient samples, that diabetes can lead to elevated circulating MtDNA. As MtDNA resembles bacterial DNA and is largely un-methylated, it can elicit an inflammatory response via activation of the TLR9 pathway resulting in inflammation. The student will investigate the hypothesis that damaged MtDNA in circulation is a direct cause of chronic and persistent "sterile inflammation" in DN. A combination of renal cell lines, animal models and patient samples will be used to determine the relationship between MtDNA, inflammation, and metabolic function, and develop strategies to prevent MtDNA induced damage. Specifically the following goals will be met.

1. Isolate circulating MtDNA from patients, rodent models and cells, and measure quantity/ quality and association with diabetes, inflammation and metabolic function.
2. Set up in-vitro systems for measuring MtDNA induced inflammation and test a range of TLR9 pathway inhibitors to prevent inflammation.
3. Test a range of compounds for the sequestration/ removal of damaged MtDNA from circulation. This work could lead to novel treatment strategies for DN.

The student will join a vibrant research group and gain expertise in numerous molecular biology/cell biology techniques including real time qPCR, ELISA, immuno-histochemistry, tissue culture, cellular bioenergetics, as well as experiencing translational approaches in an exciting and emerging research area addressing strong unmet clinical needs.

Two representative publications from supervisors:


Malik, A.N & Czajka, A. Sep 2013 “Is mitochondrial DNA content a potential biomarker of mitochondrial dysfunction?” Mitochondrion. 13, 5, p. 481-492
K38.1 p38α-TAB1 interaction: a model system to study scaffold mediated autophosphorylation in kinases and its role in ischemia and senescence.

Co-Supervisor 1: Dr Gian F. De Nicola
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Project description:
The alpha isoform of p38 mitogen-activated protein kinase (p38α) is expressed in all cells and is involved in multiple stress-sensing pathways. The purpose of this studentship is to examine p38α auto-activation. This atypical mode of activation was first described in the heart during ischemia where p38α binds a scaffold protein known as TAB1. In our laboratory we have solved the X-ray structure of the complex between p38α and TAB1. The structure has revealed the molecular details of the auto activation reaction, it also showed that the binding surface between the two proteins is conserved across species suggesting a role for the interaction that goes beyond the heart and ischemia. Recent data in the literature show that p38α-TAB1 interaction regulates the ubiquitination pathway and the senescence phenotype in T-cells.

To further characterize this interaction we have created a knock-in mouse where the binding between the two proteins is selectively abolished by mutating four key residues on TAB1 that are responsible for p38α-TAB1 recognition.

The purpose of the studentship is to use the knock-in model as a tool to investigate in vivo the role of the interaction in ischemia, in the ubiquitination pathway and in the senescence phenotype.

A second aspect of the project involves screening for small molecules that inhibit the interaction between p38α and TAB1. Isothermal calorimetry, NMR spectrometry and X-ray crystallography will be used to assess the affinity and the mode of action of any potential hits.

The laboratory of prof Marber is where the cell biology and mouse physiology side of the project will be carried out whereas the biophysical and structural biology side of the project will be carried out in the laboratory of dr De Nicola.

Year 1-2: Characterization of the knock-in mouse model and biophysical characterization of small molecules inhibitors of the p38α-TAB1 interaction.
Year 3-4: Potential p38α-TAB1 inhibitors tested in ex-vivo models

Two representative publications from supervisors:

http://www.nature.com/nsmb/journal/v20/n10/full/nsmb.2668.html
http://circ.ahajournals.org/content/126/3/357
K39.1 Elucidating the crosstalk between lymphocytes and intestinal epithelial cells using human mini-guts.

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Co-Supervisor 2: Dr Joana F Neves (Basic Scientist)
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Project description:
Studies, by us and others, have found that maintaining intestinal homeostasis depends on the interactions between the gut epithelium, the intestinal microbiota and the gut-- - associated immune system. Disrupting this delicate balance usually results in intestinal inflammation, which is associated with several diseases such as Inflammatory Bowel Disease (IBD) and cancer.

We recently developed a novel in vitro system of lymphocyte culture in intestinal organoids ( “mini-guts” ) that mimics the intestinal environment. This pioneer system allows us to identify and dissect the mechanisms that govern the crosstalk between lymphocytes and epithelial cells at the intestinal barrier. Certain populations of a recently discovered group of lymphocytes, called Innate Lymphoid Cells (ILC) are increased in IBD patients. Thus, we are particularly interested in studying the interaction of ILC with intestinal epithelial cells.

Aim-1) Ascertain the differences between intestinal organoids established from inflamed and non-inflamed tissue of IBD patients and their effect on the function and differentiation of ILC (Years 1-2). Aim-2) Understand the effect of ILC on the biology of intestinal epithelial cells (Years 2-3). Aim-3) Identify the molecular pathways that regulate the crosstalk between ILC and intestinal epithelial cells (Years 3-4).

This study can lead to the identification of novel targets to modulate ILC and intestinal epithelial cells in order to promote intestinal homeostasis.

During this project the student will acquire a wide range techniques such as flow cytometry, imaging, molecular biology (including CRISPR), transcriptomic, bioinformatic and lymphocyte biology and mucosal immunology techniques (mouse/human). The supervisors and their established collaborations have vast expertise these areas.

Two representative publications from supervisors:


*These authors contributed equally to this work.
K40.1 miR-acles in collecting ducts underlie kidney scarring driven by risk factors

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Project description:
Why do albuminuria, hypertension and excitation of renal nerves cause kidney scarring in chronic kidney disease (CKD)? Our overarching hypothesis is that collecting ducts (CDs) play a central role in mediating the damage via a final common pathway.

We found that retinoic acid (RA) activity is physiologically confined to CDs and that albuminuria sequesters RA, significantly reducing RA activity in CDs in CKD mice (Figure 1).

In cultured CD cells, our microarray studies have identified miR29b, miR30e and miR140 as the top three microRNAs (miRs) most suppressed by inhibiting RA biosynthesis and antagonising RA receptors. These 3 miRs directly repress expression of down-stream effectors and premier mediators of fibrogenesis, including collagens, Ctgf and Smad3.

We hypothesise that urinary albumin, renal neurotransmitters and mediators of hypertension repress expression of RA/RAR-dependent miR29b, miR30e and miR140, and thus cause kidney fibrosis (Figure 2).

We will address this hypothesis mainly in cultured CD cells and renal fibroblasts. CD-derived urinary exosomes and kidney tissue already collected from CKD patients with and without the concerned risk factors will also be examined.

Year 1: Optimising cellular models (including CD-derived mesenchymal stem cells) for studying the proposed albuminuria-RA/RAR-miRs axis; effects of albumin, neurotransmitters and mediators of hypertension on RA/RAR activity in CD cells; pilot studies of urinary exosomes;

Year 2: Stable transfectants of pre-miR, anti-miR or negative control miRs and effects on fibrogenesis; CD-derived urinary exosomes;

Year 3: miR target gene analysis and mechanisms of action in cellular models, including CD-derived mesenchymal stem cells.

Year 4: Animal models to validate in vitro findings and to explore intervening new strategies.

Two representative publications from supervisors:


K41.1 Evaluation of PSK kinases as suitable targets for breast cancer treatment

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Project description:
Drugs that target and inhibit microtubule dynamics (eg. taxanes) provide one of the most effective classes of therapeutics for the front-line treatment of metastatic breast cancer, but these compounds also produce debilitating side effects and patients relapse and become resistant within 3-8 months. Microtubules are therefore proven targets for chemotherapy and their disruption works well in the clinic, but additional and better strategies are now needed to target these structures in dividing breast cancer cells to provide more effective and longer lasting treatment.

In seeking candidates for intervention we have identified an unusual family of proteins called prostate-derived sterile 20-like kinases (PSKs), which bind microtubules and regulate their stability and organisation. PSKs are activated catalytically in dividing breast cancer cells and this activity is required for their proliferation.

The initial project will use novel small molecule inhibitors for PSKs to assess the requirements for these proteins during cell division and migration.

The PhD objectives will be to:

• Identify biological functions for PSKs and their mechanisms of action in breast cancer cells
• Inhibit PSK activity and downstream substrates in breast cancer cell models in order to alter cell proliferation and migration
• Characterise novel chemical inhibitors for PSKs and establish their biological effects on malignant cells
• Use breast cancer tissue arrays to identify patient subtypes suitable for kinase inhibition and therapy

The results will establish whether PSKs offer suitable targets for breast cancer therapy.

Skills:
• Expertise in cellular and molecular biology techniques (Morris)
• Knowledge of cancer cell biology and treatment (Papa)

Two representative publications from supervisors:

Wojtala RL, Tavares IA, Morton PE, Valderrama F, Thomas NS, Morris JD. Prostate-derived sterile 20-like kinases (PSKs/TAOKs) are activated in mitosis and contribute to mitotic cell rounding and spindle positioning. 2011. J. Biol. Chem. 286. 30161-70.

K42.1 Defining the role of RhoE in fibrotic skin disease

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Project description:
Dermatofibromas are benign, collagen-dense skin growths that can occur at any skin site. Most dermatofibromas are solitary and sporadic; a few are multiple and familial. Despite affecting ~3% of the population in the UK, the mechanisms that lead to these growths are currently unknown. Using exome sequencing, we have recently identified a novel heterozygous mutation in patients with autosomal dominant multiple familial dermatofibromas within RND3 that encodes the GTPase protein RhoE (mutation: p.Thr231Met). RhoE is known to be an important regulator of the actin cytoskeleton in a range of cell types but mutations in this protein have not been previously reported in any genetic human disease. Moreover, the potential involvement of RhoE in the development of fibrosis has not been previously proposed. Our preliminary evidence suggests that the putative dominant-negative missense mutation results in a loss-of-function of RhoE, leading to increased nuclear RhoE levels, altered organisation of F-actin in fibroblasts and potential changes in proliferation, mechanosensing and contraction. The aim of this study is to unravel to role of RhoE in controlling human dermal fibroblast growth and collagen synthesis. The project will employ a range of biochemical, biophysical and advanced microscopy techniques to study RhoE function in isolated fibroblast cells and 3D extracellular matrix and dermal equivalents. The specific aims are:
- Generate inducible stable fibroblast line to express controlled levels of wild-type or mutant RhoE-GFP. Analyse levels of WT and mutant RhoE in the nucleus vs cytoplasm, and determine the role of the mutation p.Thr231Met on proliferation.
- Determine whether p.Thr231Met leads to aberrant collagen synthesis/assembly in 2D and 3D models. Monitor cytoskeletal dynamics, cell growth and ECM assembly over time using live cell time-lapse fluorescence microscopy. Define how mutation of RhoE contributes to the balance of intracellular and extracellular force production in fibroblasts.
- Investigate the mechanisms controlling RhoE nuclear shuttling and define how modulation of nuclear localisation regulates transcriptional changes leading to fibrotic disease.

Data arising from this study will provide novel insight into the regulation of fibrosis and dermatofibroma development in the skin with broad-reaching implications for potential drug development in other fibrotic diseases.

Two representative publications from supervisors:

Taming neutrophil responses to human myocardial infarction as a therapy for heart failure.

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Project description:
Heart failure (HF) is a major global concern that places significant financial impact on health services. Patients who survive myocardial infarction (MI) are likely to develop HF in later life, yet there is an unmet need to develop therapies aimed at reducing this risk. During the first 24 hours following MI, neutrophils infiltrate the infarcted myocardium and home to necrotic cardiomyocytes and mediate further tissue damage by releasing matrix-degrading enzymes and reactive oxygen species (ROS). Although such aggressive neutrophil responses evolved to curb the spread of an infection, excess neutrophil activity in the context of MI can promote adverse cardiac remodelling that contributes substantially to HF. We hypothesise that blocking neutrophil homing to the infarcted heart, particularly within the first 24 hours of MI, would greatly improve patient outcome. This project aims to target the cell adhesion molecule, L-selectin, on neutrophils and determine its contribution to homing towards necrotic cardiomyocytes. We hypothesise that targeting L-selectin in neutrophils disturbs their polarity and migration towards necrotic cardiomyocytes, which will minimise the release of matrix-degrading enzymes and ROS.

Workflow
Year 1 – Generate lentiviral expression vectors (for WT and mutant L-selectin), engineer neutrophil cell lines to perfuse into flow chambers. Culture cardiomyocytes, induce necrosis, and incorporate necrotic cells into flow chambers to model MI in vitro. Perfuse neutrophils into flow chambers containing necrotic cardiomyocytes.
Year 2 – Perform time-lapse imaging of neutrophils responding to necrotic cardiomyocytes. Compare neutrophil behaviour in cells expressing WT vs mutant L-selectin.
Year 3 – Optimise imaging for release of neutrophil azurophilic granules and nuclear extracellular traps (NETs) to gain insight into neutrophil physiology. Compare WT with mutant cell lines.

Students will receive training in: microscopic imaging, building and performing flow assays in microfluidic devices, culture of primary endothelial cells and cell lines, lentiviral expression vector design and production, genetic engineering of cell lines, and isolation of primary neutrophils from whole blood.

Two representative publications from supervisors:


K45.1 Studying the immune infiltrate in metastatic tumours in the search of new biomarkers and therapeutics

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Project description:
Metastasis accounts for >90% of cancer-related deaths. Detachment of tumor cells from a primary site, cell migration, transport through vessels and escape from immune-surveillance are part of the metastatic process. We aim to understand the connections between cancer migratory potential and escape from immune surveillance. Metastatic dissemination is controlled by Rho-ROCK and JAK-STAT3, two pathways that control cell migration and immunomodulation respectively.

On the other hand, activation of immune responses as a therapy in cancer holds promise, but only a proportion of patients respond to current treatments. Therefore, there is an unmet need for new therapeutic strategies that effectively activate immune responses in the majority of patients. We also need to understand how cancer cells interact with immune cells in late stages of metastasis. Preliminary work from our group shows that metastatic melanoma cells secrete higher levels of immunomodulatory cytokines than non-metastatic counterparts. Such cytokines are associated with aggressive metastatic behaviour in human melanoma patients. Therefore, in this project we aim to investigate if not only metastatic melanoma cells, but also other metastatic cancer cells have immunomodulatory potential. We propose to answer the following questions.

Year 1. Since macrophages can propel metastasis to distant organs, can secreted factors from highly metastatic cancer cells affect monocyte recruitment and macrophage differentiation?
Year 2. Can immune-modulatory cytokines from highly metastatic cancer cells be used as biomarkers with prognostic value for cancer patients?
Year 3 (and 4 when applicable). Can we use inhibition of migratory potential as a way to activate a more efficient immune response against a tumour? We will study the possible interactions between macrophages, T-cells and cancer cells

This studentship covers basic and translational biomedical research, including acquisition of multi-disciplinary experience in cell biology, biochemistry, state-of-the-art imaging and patient data analysis.

Two representative publications from supervisors:


K46.1 Mutations and mechanisms in cholestatic liver disease; using Whole Exome Sequencing and in vitro models

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Project description:
The project will combine the identification of new disease genes, and the in vitro investigation of the consequences of mutations in those genes.

We have identified several new causes of liver disease, and have others to find. This component of the work will involve the identification of suitable patients and samples, and the analysis of whole exome and whole genome data. Over 500 patients with cholestatic liver disease are available for testing. The feature common to all is failure of normal bile formation. This work will lead to the identification of new disease genes. Equally important is the understanding of the mechanisms by which mutations lead to disease. Previously the investigation of mutations has been through mRNA analysis, Western Blotting, immunohistochemistry and in vitro expression. Currently in vitro models are being used, including gene knockdown in human hepatocytes and cell lines. This is being expanded to include the study of hepatocyte-like cells derived from induced pluripotent cells generated from patients.

Initially the student will analyse whole exome data for new disease causing mutations and work with a post-doctoral scientist in the study of cells for in vitro studies. They will learn the principles of next generation sequence analysis, cell culture, immunohistochemistry and the examination of protein distribution in normal and abnormal cells.

The project will be far reaching. Professor Thompson will bring the genetic expertise, Dr Deheragoda that of histology, immunohistochemistry and electron microscopy. Other work will require assistance from established collaborators within KCL, London, USA and Canada.

Two representative publications from supervisors:


Unravelling the drivers of dysregulated T cell responses in Inflammatory Bowel Disease

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Project description:
Inappropriate activation of CD4+ T-cells plays a central role in immune-mediated diseases, such as Crohn’s disease (CD). We have demonstrated an unexpected role for the evolutionary conserved complement system (autocrine signalling of CD46 and C5a receptors on T cells) and the NLRP3 inflammasome in the differentiation and effector function of CD4+ T-cells. We will now test the hypothesis that this exciting novel activation pathway is dysregulated in CD - a prototypical T-cell mediated inflammatory disease. Specifically, we will determine whether CD46/C5aR activation of gut CD4+ and/or CD8+ T-cells triggers inflammasome activation and augmented pathogenic cytokine production in CD, and whether inflammasome inhibition supresses T-cell derived pathogenic cytokine production.

Skills/training:
Isolation/functional analysis of T-cells from CD patients
Molecular/immunological methods (cell culture, gene array analyses, flow cytometry, CyTOF, confocal microscopy, Image Stream, cytokine measurement, cloning, Western blots, etc.)
Successful candidates will also become key members of our integrated clinical/academic inflammatory Bowel Disease research team at Guy’s and St Thomas’ Hospital (with one of the largest/research active IBD clinics in the UK).

3-year plan:
Defining the contributions of CD46/C5aR/NLRP3 inflammasome activation in normal Th1/Th17 CD4+ and CD8+ T-cell responses (healthy donors, blood-derived) – Year 1-2
Defining the expression of complement receptors and the inflammasome in intestinal resident CD4+ and CD8+ T-cells, and other key immune cells (including innate lymphoid cells) from non-inflammatory control patients – Year 1-3
Assessing whether alterations in complement/inflammasome signals contribute to dysregulated immune responses in CD and ulcerative colitis patients, and whether complement/inflammasome inhibition attenuates pathogenic responses – Year 1-3.

Two representative publications from supervisors:

K48.1 Mast cells in food allergy

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Co-Supervisor 2: Dr Alexandra Santos
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Project description:
Allergic reactions to food can range in severity from mild local symptoms to severe anaphylaxis. Peanut allergy is particularly troublesome, with patients often reacting to small amounts of the allergen with symptoms that can be life-threatening. It is not known why only some of the allergic patients develop severe reactions.

Mast cells (MCs) are a major cell type involved in acute allergic reactions to foods and anaphylaxis. Tissue mast cells derive from circulating progenitor cells that remain uncharacterised. Using microarray screening we identified a set of surface markers allowing for a potential identification of human MC progenitors. Our aim is to validate the markers, isolate circulating MC progenitors and define their transcriptional signature in order to characterise receptors responsible for MC progenitor migration to tissues. Migration and differentiation of MC progenitors into anaphylaxis-related phenotype will be studied in patients with peanut allergy and in non-allergic individuals.

The successful student will acquire theoretical and practical skills in molecular biology of mast cells (cell isolation and culture, flow cytometry), functional genomics (microarrays, RNAseq), cell signalling, and molecular immunology (RT-PCR, siRNA, lentiviral overexpression) as well as translational research skills in allergy and clinical immunology.

Year 1: Identification of markers characteristic for human circulating mast cell progenitors, in vitro mast cell differentiation models

Year 2: Defining the molecular fingerprints for MC progenitors, cell migration

Year 3 + 4: Studying MC progenitors in patients with allergy to peanuts and controls; characterisation of anaphylaxis-related mast cell phenotype in patients with peanut allergy

Two representative publications from supervisors:


K49.1 Resolving genetic insights into T-cell biology to understand susceptibility to inflammatory bowel disease

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Project description:
Inflammatory bowel disease (IBD) is one of most complex genetic diseases known to man, with genetic variants at over 200 genetic loci associated with altered disease susceptibility. Importantly, most of the genes associated with IBD encode proteins involved in the immune response. The functional repercussions of these associations will be defined in this project by comparing immune cells from IBD patients with and without defined IBD-associated variants. Data generated in this study will provide unique insights into the cellular and molecular mechanisms of inflammatory disease and guide the search for new therapeutic targets.

Skills/training:
• Isolation and functional analysis of T-cells (and other key immune cells from IBD patients): cell culture, flow cytometry, CyTOF, gene cloning, western blotting, etc.
• Cutting edge genomics training, including RNA sequencing and bioinformatics analysis.
• Opportunities to work with in vivo inflammatory disease models
• Successful candidates will also become key members of our integrated clinical/academic IBD research team at Guy’s and St Thomas’ Hospital (with one of the biggest IBD clinics in the UK).

3-year plan:
- Use novel fine-mapping, whole genome sequence data and tissue-specific gene expression analyses to identify likely causal variants. (Years 1-2)
- Determine how the function of regulatory (Tregs) and effector (Th1, Th17) T-cell lineages (and other key immune cells) differ in IBD patients harboring specific variants at IBD-associated (e.g. SATB1, IL2RA, IL23R). (Years 2-3).
- Use In vitro and in vivo inflammation models to determine how these mutations functionally impact disease manifestations (year 3-4).

Two representative publications from supervisors:


K50.1 Dissection of the molecular interplay between skin cells and the resident microbial communities in skin autoimmune disease

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Co-Supervisor 2: Dr Sophia Tsoka
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Name of Collaborating Clinician: Prof. Frank Nestle
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Project description:
The Human Microbiome Project has estimated that the microorganisms living in humans outnumber human somatic and germ cells by a factor of ten. The genomes of this microflora, which has co-evolved with humans, provide an additional source of information to explain human diseases. Some of these microbes have been shown to create metabolites that interfere with the expression of genes associated with autoimmune disease, suggesting their role in the etiology of several autoimmune conditions.

Our proposal focuses on the analysis of microbial communities and their interaction with the host environment in skin samples from patients affected by the skin’s autoimmune frequent conditions of psoriasis and atopic dermatitis. The project leverages on the large public and proprietary data sets generated from on-going collaborative research on skin’s conditions. These encompass host data - skin’s gene-expression, patients’ genetic profiles and immune-phenotyping - as well as skin metagenomics profiles obtained from NGS sequencing.

The project is organized in two main and closely inter-connected streams:
1) Application and extension of existing methods to detect associations between hosts’ and metagenomics’ profiles and clinical/pathological patients’ features (years 1-2)
2) Development of a systems biology analytical framework based on correlation- and Bayesian-based networks to dissect statistical associations and causal relationships between host skin’s properties and microbial communities. Examples are the identification of host SNPs associated with the abundance of microbial species or microbial pathways, and the influence of altered microbial ecosystems on skin’s gene expression and immune phenotypes (years 3-4).

Results will contribute towards the reconstruction of the molecular portrait of host/microbial molecular perturbations occurring in chronic inflammatory skin disorders, at the same time establishing the bases for biomarker discovery and therapeutic intervention.

Two representative publications from supervisors:


K51.1 Virus-host interactions in chronic viral hepatitis

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Project description:
With over 500 million people chronically infected globally, the hepatitis B (HBV) and C (HCV) viruses are major health burdens. Despite the availability of a prophylactic vaccine for HBV and potent antiviral drugs for HCV, approximately 4 million new cases are reported annually for each infection and the burden of disease is expected to rise over the next years. These viruses are the main cause for hepatocellular carcinoma (HCC), the fifth most common cancer worldwide.

Our research focuses on understanding the complex virus-host interactions underlying these chronic infections and the role they play in the pathogenesis of the associated liver diseases (1,2).

We demonstrated that infectious HCV particles are coated with human apolipoproteins that play key roles in viral attachment and entry (1) and incorporate a range of cellular proteins required for infection (PNAS, under revision). Moreover, we have been studying how antiviral therapies elicit both immune responses and viral evasion countermeasures in HBV infection (2). Therefore, our expertise places us in a unique position to interrogate the role of host factors in the propagation of HCV and HBV infections in vitro as well as in patients.

The Aims of this project are to:
1. Characterize the role of recently identified new virus-associated host factors in HCV infection
2. Screen for novel cellular factors required for HBV propagation
3. Compare the expression levels and genetic variation of cellular factors in HCV/HBV-infected patients responding, or not, to therapy to determine whether they can be used as biomarkers to predict clinical outcome.

Two representative publications from supervisors:


K52.1 T cell polarization by monocyte-derived dendritic cells

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Project description:
Mononuclear phagocytes include monocytes, macrophages and dendritic cells (DCs). DCs features include a strong endocytic/phagocytic ability underlying their ability to capture antigens to migrate from peripheral tissues to the T cell zones of lymphoid organs and to present antigens to T cells by MHC class I and II molecules.
DCs represent a distinct lineage characterized by its dependency on the Flt3L growth factor engaging the Flt3 receptor tyrosine kinase. Fate mapping experiments and specific molecular markers define DC as a specific hematopoietic lineage.
Functional studies in mice based on conditional ablation of DCs in vivo have shown that they control the activation of some CD4+ Th1 cells upon inflammatory challenge. Conversely, conditional ablation of monocytes and macrophages leaving DCs unaffected impairs partially the activation of CD4+ Th1 cells in inflammatory conditions. These experiments led to hypothesize that a fraction of mononuclear phagocytes distinct from “classical” DCs participate significantly to the generation of CD4+ T cell responses during immune responses.
Although multiple in vitro studies using both mice and human cells suggest that non-DCs phagocytes can acquire, to some extent, some DC phenotypic and functional features under inflammatory conditions, the physiological role of these so-called “inflammatory DCs” is not clearly defined.
This project intends to characterize the ability of inflammatory, human monocyte-derived DCs to drive the polarization of naive CD4+ T cells and to drive the re-stimulation of already polarized CD4+ T cells (Th1, Tr1, Th17, Tregs). Ultimately this research should highlight the role inflammatory DCs play in pathogenic T cell responses that contribute to chronic inflammatory diseases such as rheumatoid arthritis.
Skills/techniques include: human and mouse immune cell isolation/culture, multi-colour flow cytometry, cytokine detection ELISA, Luminex, intracellular staining), differentiation of human monocytes, T cell polarization assay, cellular immunology.

Two representative publications from supervisors:


TNF-α blockade induces IL-10 expression in human CD4+ T cells.
K53.1 Molecular targeting of prostate cancer invasion

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Project description:
New drugs to target prostate cancer (PC) metastasis are urgently required. Cancer cells are thought to use invadopodia to invade normal tissue and we have established that PC cells make invadopodia. We have previously shown that a “drugable” protein kinase PAK4 is a critical driver of PC cell migration and now have data linking PAK4 to invadopodia. However, there is much we don’t understand about PAK4 in invadopodia, invadopodia in PC, and how these processes are regulated/could be targeted. This project aims to enhance our understanding using cell biology, biochemistry and high resolution microscopy (HRM) techniques. Ultimately, we will test our findings in patient material (Rudman Lab in collaboration with Van Hemelrijck lab KCL). The Wells lab has a plethora of expression constructs, unique PAK4 specific inhibitors (PAK4i) and human tissue validated antibodies.

Project Outline: Suitable for 1+3 or 4 yr programme

Rotation: screen PC cells and our patient derived cells for invadopodia. Test PAK4i treatment on invadopodia.

Y1: Optimise PC cell lines for quantitative invadopodia analysis. Develop 3D matrix invasion assay. Correlate invadopodia formation with invasion. Use PAK4CRISPR constructs (in Wells Lab) to generate PAK4 null cells (PAK4CR).

Y2: Generate PAK4-tagged (and mutant PAK4) knock-in PAK4CRISPR (PAK4KI) cells; use to identify PAK4 protein partners. Use proximity ligation assays and HRM to identify invadopodia binding events.

Yr3/4: Continue to explore PAK4 activity: regulators/substrates. Use PAK4CR cells, PAK4KI cells and PAKi to investigate how PAK4 kinase activity mediates PC invadopodia formation/3D matrix invasion. Test key findings in clinical material.

Two representative publications from supervisors:


54.1 Adeno-associated virus-host interactions and their impact on gene therapy vector production.

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Project description:  
Adeno-associated virus (AAV) is a human dependovirus that appears to have evolved an optimal working relationship with host and helper virus. With the support of viruses such as adenovirus or herpes virus, AAV replicates successfully, causing a cytopathic effect only in those cells which were already destined to suffer from helper virus infection; in the absence of co-infection the virus remains latent. AAV’s wide-ranging activities, from replication, packaging to integration, are controlled by the Rep proteins. Given the multitude of functions of the Rep proteins in the viral life cycle it is believed that AAV, like other more complex DNA viruses, interacts with cellular proteins to fulfill its many functions and to stage an ideal environment for viral replication. We recently employed a new technique for proximity-dependent labeling of proteins in eukaryotic cells, termed BioID, to screen for physiologically relevant protein interactions with Rep. We have successfully validated one of the candidates and have discovered that Rep interacts with the candidate to overcome its repressive effect on viral transcription. This project is designed to further understand the intricacies of the observed virus-host interaction (year 1 and 2) and to investigate how this factor inhibits recombinant AAV production (year 2 and 3). AAV has become a frontrunner as a vector for human gene therapy based on results from a number of clinical trials. Knowledge gained from this proposal could be exploited to help overcome the challenges of production for wide clinical use. The student will learn various molecular virology and cell biology techniques and will have the opportunity to communicate his/her research in an academic as well as a commercial setting.

Two representative publications from supervisors:


Contribution of canonical Wnt signalling to pathology in Facioscapulohumeral muscular dystrophy

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Project description:
Skeletal muscle is composed of muscle fibres, which are maintained and repaired by stem cells called satellite cells. This efficient mechanism however, gradually fails in muscle wasting diseases such as muscular dystrophies. We have found that Wnt signalling is perturbed in Facioscapulohumeral muscular dystrophy (FSHD) (1), and that Wnt signalling controls muscle stem cell function (2) and neurogenesis (3).

This project will investigate whether manipulation of Wnt signalling by targeting signalling pathway proteins (e.g. DVL1-3 and axin1/2) affects muscle stem cell migration to promote effective regeneration using human (1), mouse (2) and zebrafish (4) FSHD models.

Objectives:
Year 1: Measure Wnt signalling genes (e.g. β-catenin, axin1/2, DVL1-3) and Wnt activity in FSHD cells and models.
Year 2: Determine how manipulation of Wnt signalling affects muscle formation in FSHD cell and animal models using drugs, gene-knockdown and over-expression.
Year 3: Identify genes responding to Wnt signalling in models of FSHD using transcriptomics and bioinformatics.
Year 4: Characterise effects of Wnt signalling manipulation on muscle stem cell behaviour using multiphoton imaging in vivo (zebrafish) and in vitro (mouse).

Skills training includes: Molecular Biology (e.g. cloning), Cell Biology (mouse/human cell culture, retroviral-infection, SiRNA-mediated gene-knockdown), Animal Models (mouse, zebrafish), Gene Expression/Protein Analysis (quantitative PCR, Western blotting, immunostaining) and Imaging/Time-Lapse using state-of-the-art confocal and multiphoton microscopy.
   Zammit: expertise in muscle stem cell function in health and disease.
   Knight: expertise in zebrafish models and imaging (4)
   Norwood: clinician specialising in neuromuscular disease.

Two representative publications from supervisors:

K56.1 Defining the immunological processing of preproinsulin to T cells in autoimmune diabetes

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Project description:
Autoimmune disease affects ~5% of the population. Yet we understand very little about how and why the immune system targets self-antigens. Type 1 diabetes (T1D) is a chronic, complex autoimmune disease in which islet beta-cells are destroyed by the immune system, leading to insulin-deficiency. In T1D we know some of the self-antigens that are targeted (eg preproinsulin). This project will focus on understanding how preproinsulin is displayed to the immune system at a molecular and cellular level in patients who develop T1D.

The project involves studying the intracellular, molecular pathways (antigen-presentation, antigen-cross-presentation) through which preproinsulin is displayed on HLA-molecules to T-lymphocytes. Antigen-presenting-cells (APCs) will be manipulated genetically to express antigens/HLA-molecules of interest. Preproinsulin presentation will be studied using proteomics. APCs will be manipulated using gene-editing to knock-out/enhance enzymes involved in antigen-presentation to pinpoint relevant immunological processing pathways. Presented preproinsulin peptides will be examined for recognition by the immune system of T1D patients using peripheral-blood immune cells and advanced technologies (pHLA-tetramers and multi-dimensional flow-cytometry).

Translational aspects of this project centre on studying patients to identify disease biomarkers of use in the clinic to monitor patients undergoing immunotherapy for T1D, or as tools for prediction of disease progression.

3+1 configuration:
Year-2: Antigen-presenting cell culture, transfection of genes into antigen-presenting-cells, gene-editing (CRISPR), proteomics studies
Year-4: Translation: study T1D patients and target biomarker profiles.

Two representative publications from supervisors:


K57.1 Identification and validation of driver genes in oesophageal adenocarcinoma

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Supervisor 2: Professor Jesper Lagergren
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Name of Collaborating Clinician: Jesper Lagergren

Project description:
Oesophageal adenocarcinoma (OAC) is characterised by poor prognosis and rapidly increasing incidence, with the highest incidence globally in the UK. This project aims to analyse whole genome sequence (WGS) data of several hundred OACs to predict mutated genes that drive or co-drive OAC development. Predicted drivers will be experimentally validated using a combination of cell-based and tissue-based assays.

WGS data derive from ICGC and OCCAMS, a large UK network recruiting OAC patients for tissue and clinical data collection. St Thomas’ Hospital is a key centre in OCCAMS under supervision of Prof. Lagergren. This provides unique opportunities to test predicted driver genes on OAC samples where they are found altered. It also allows relating genomics findings with clinical factors, including the translational aspect how identified driver genes influence prognosis.

In year one, the student will learn computational biology techniques, mainly for analysis of next generation sequencing data (alignment, quality control, variant calling and annotation).
In year two and three, the student will apply the computational approach developed in Ciccarelli’s group (D’antonio&Ciccarelli Genome Biol 2013) to predict sample-specific cancer drivers in OAC.

In year three and four, the student will validate predicted drivers by perturbing the genes and measuring the effect of this perturbation on cell growth. Gene perturbation will be performed through gene editing, RNA interference and gene overexpression, depending on whether the gene is predicted to have oncogenic or tumour suppressor activity. The student will also verify abnormal staining and overexpression of the mutated proteins in cancer tissue blocks.

Two representative publications from supervisors:

D’antonio M, Ciccarelli FD Integrated analysis of recurrent properties of cancer genes to identify novel drivers 2013, Genome Biology 14:R52

Wnt signalling in human prostate cancer stem cells: a target for therapy

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Project description:
It is now thought that cancer is a disease of stem cells where cancer stem cells (CSCs) drive tumor growth and relapse. It follows that if CSCs could be targeted and eliminated, a curative therapy for cancer could be formulated. The Wnt signalling pathway plays a key role in stem cells self-renewal and differentiation and in carcinogenesis of prostate, penile, colon and breast cancers. Wnt signalling is transduced by two key transducers, intracellular free calcium ([Ca2+]i) and β-catenin, a potent transcription factor co-activator. We have shown that the activation of Wnt signalling first increases [Ca2+]i that depolarizes the cell and nuclear electrical potential to facilitate β-catenin translocation into the nucleus to activate gene (including numerous proto-oncogenes) transcription. We hypothesize that the self-renewal of CSCs is regulated by Wnt signalling mechanism. We further propose that targeting the Wnt signalling pathway in CSCs could provide an avenue for therapy. To do this we need to understand the role of electrogenic Wnt signalling in human CSC.

The purpose of this project is to isolate human CSCs from prostate tissue and investigate the characteristics of Wnt signalling by:
1. [Ca2+]i release and cell membrane currents by simultaneous patch clamp electrophysiology and live calcium imaging using multi-photon confocal microscopy
2. β-catenin translocation into the nucleus using immunocytochemistry
3. assessing self-renewal of CSCs ± Wnts using in vitro cell culture
4. using human models of cancer in mice

At the end of this project we hope to identify CSCs that could be targeted for cancer therapy.

Two representative publications from supervisors:


Investigating novel pathways in lymphoma

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Name of Collaborating Clinician: Dr Richard Dillon,
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Project description:
Diffuse large B-cell lymphoma (DLBCL) is the most common form of haematological malignancy. A large proportion of these cancers are driven by deregulated expression of Bcl6 (B-cell lymphoma protein 6). Bcl6 can function as a transcriptional repressor by remodelling the epigenetic environment at its target genes. However, our understanding of the molecular mechanisms underpinning its functions remains limited and recent studies show this protein also functions in alternative, as yet unknown pathways.

We have identified a previously uncharacterised mammalian protein that associates with Bcl6 at a large proportion of its binding sites in the genome, and preliminary data suggest this factor functions together with Bcl6 in a pathway that is distinct from the previously described gene repressive roles. The student will use a combination of molecular biology and computational techniques to carry out a functional study of this newly uncovered interaction. Specifically, the student will use CRISPR to knock out the novel Bcl6-associating factor in normal and malignant cells, and subsequently analyse the changes in transcription and epigenetic modifications genome-wide using RNA-seq and ChIP-seq, respectively. Ultimately the aim is to understand the functional relationship between Bcl6 and its newly identified interactor in the context of normal and malignant development. This will improve our understanding of the mechanisms underpinning the functions of Bcl6 and could also potentially point out specific strategies to develop novel therapeutic interventions against a subset of lymphomas.

Two representative publications from supervisors:


K60.1 Role of Tregs in modulating liver inflammatory disorders

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Co-Supervisor 2: Professor Alberto Sanchez-Fueyo
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Project description:
The liver is constantly exposed to food antigens and low levels of endotoxin from the gut via the portal vein. As a result, it has developed mechanisms to suppress immune responses elicited by these antigens whilst maintaining the capacity to respond to harmful stimuli such as infectious agents. Regulatory T cells (Tregs) are essential for the maintenance of peripheral self-tolerance and immune homeostasis in various tissues, and are essential to prevent autoimmunity. Tregs are present in the human liver and are known to influence the outcome of liver inflammatory disorders. However, the factors that modulate Treg homeostasis in the liver, and whether this is influenced by the cytokine microenvironment, are unclear. In addition, the extent to which acute and chronic liver diseases influence Treg function remains to be defined.

Objectives:
1. Phenotypic and functional characterization of intrahepatic Tregs in humans and mice (Year 1)
2. Assessment of liver resident Tregs stability in the presence of pro-inflammatory cytokines in a murine model of liver fibrosis resolution (Year 1).
3. Characterization of the effects of IL-2 therapy on Treg function and trafficking during liver inflammation (Year 2).
4. Role of Tregs in liver tissue regeneration and immune response resolution (Year 2).
5. Characterisation of Treg phenotype and function in patients with different degrees of liver failure. And correlation with experimental animal data (Year 2-3).

Skills training: animal models of liver inflammation and fibrosis; cell isolation & culture (human and mouse, MLR, Treg suppression assay); cell biology (flow cytometry, ELISA); molecular biology (real-time PCR, cDNA microarray, DNA methylation analysis).

Two representative publications from supervisors:


K61.1 Molecular mechanisms in B cell class switching to IgE, plasma cell differentiation and asthma

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Co-Supervisor 2: Prof. Hannah Gould
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Project description:
Asthma and allergic diseases have reached epidemic proportions, and more effective treatments are urgently required. Pathology is caused by allergen-specific IgE antibodies, generated following allergen activation of B cells, class switching to IgE, and differentiation of these cells into IgE secreting plasma cells or memory B cells. The mechanisms that determine whether a B cell switches to IgE, rather than IgG or IgA, and specifically the net effects of proliferation, cell death and the pathway and probability of differentiation are currently unknown.

We have developed cell culture conditions to study IgE production in human B cells and established assays to probe their biological activities and transcriptomic profiles. In years one and two, naïve IgM and “antigen experienced” IgG expressing B cells will be isolated and stimulated in vitro to undergo class switching to IgE. During this process the proliferative, apoptotic and differentiation potential of these cells, and their transcriptomic profiles will be investigated over time and results using B cells from asthma patients and healthy controls compared. We have brought together international collaborators from across the UK and Australia specialising in mathematical modelling of biological systems and bioinformatics. In year 3 we will combine these specialties to investigate how the biological activities of IgE expressing cells are coordinated, what genes/proteins control these mechanisms and in turn IgE production and ultimately pathology. These experiments will provide insights into the pathogenesis and treatment of asthma and allergic disease. Knowledge of these mechanisms may facilitate the identification of new drugable targets for therapy.

Two representative publications from supervisors:

IgE responses in mouse and man and the persistence of IgE memory

Soluble CD23 Controls IgE Synthesis and Homeostasis in Human B Cells
K62.1 Genome-led therapeutic target discovery in severe inflammatory skin disease

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Project description:
Human genetics is a valuable tool to prioritise molecular targets for therapeutic drug development. This project aims to utilise large-scale genomics data across many thousands of individuals (already collected) to both characterise the genetic contributors to inflammatory skin diseases and identify molecular targets for therapeutic intervention.

Our group has been at the forefront of research seeking to identify genomic loci contributing to the genetic basis of psoriasis and acne, including recent large-scale genome-wide investigations. We are currently in the process generating genomewide genotyping data on in excess of 6,500 individuals with severe acne and 10,000 individuals with psoriasis. The proposed project will utilize these data to identify further risk loci and fine mapping of these signals. The project will employ cutting edge analytical approaches aimed at integrating these data with large publicly available genomic data and transcriptomic experiments relating to skin biology. The approach will identify putative therapeutic targets whose activity is disrupted by genetic variation that predisposes these common inflammatory disorders, highlighting critical points in the disease-causing pathway can be evaluated for therapeutic manipulation.

The supervisors will provide a world-class training in contemporary genome science, statistics, bioinformatics and provide the opportunity for interaction with research groups in academia and industry. The work will be undertaken in a multidisciplinary environment supported by core facilities and underpinned by a longstanding collaboration between geneticists and dermatologists.

Two representative publications from supervisors:


Origin of inflammatory antibody responses in inflammatory bowel disease.

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Project description:
Inflammatory bowel disease (IBD) is severe, debilitating and painful inflammation of the intestine that is a lifelong health problem for sufferers. The two subtypes, Crohn’s disease (CD) and ulcerative colitis (UC) are both common, affecting 1 in 250 of the population approximately combined. Although therapies to control the disease are becoming more effective, some patients fail to respond and there is no cure. Further research is urgently called for.

One of the major changes in the inflamed intestine in inflammatory bowel disease is the accumulation of antibody producing plasma cells. The biggest relative change in the intestine is the accumulation of plasma cells making proinflammatory IgG rather than more passive mucosal antibody IgA. The movement towards IgG production in IBD has previously been though to reflect influx of cells in response to inflammation rather than an aberrant mucosal response and largely ignored.

However, we have identified, very unexpectedly, that B cells expressing IgG are normally present in the gut but unlike their IgA expressing counterparts they do not have the homing receptors that would enable them to generate IgG producing plasma cells. This project aims to develop this finding and plot how this system might be distorted to generate an inflammatory response in the gut in IBD.

The project will analyse the properties of B cells isolated from human intestinal tissue in vitro and their migration through the gut by next generation sequencing of immunoglobulin heavy chain genes that are identifiers of clonal identity. This will determine if inflammation in IBD is driven or exacerbated by aberrant maturation and migration of previously unknown mucosal IgG responses.

Two representative publications from supervisors:
Supervisors have a long track record of collaborative translational research.


K64.1 Mechanisms of action of a novel marine natural product for the management of osteoporosis and metabolic bone disease.

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Project description:  
Natural products are chemicals from nature that can be used as the basis for new medicines. Natural products from marine sponges show exceptional promise as potential pharmaceuticals. Osteoporosis is a debilitating disease that affects millions of people worldwide. There is no cure and current treatments have many side effects. Understanding osteoporosis inhibition by natural products could offer effective strategies for the treatment of osseous deficiencies. We are working on a family of natural products called chondropsins from a Great Barrier Reef sponge. Our current data provide strong evidence that the accepted mechanism of action of the chondropsins is incorrect. The prospects of using chondropsins as tools to manipulate bone cell activity with a view to provide mechanistic data that underpin development of a new medicine is completely novel and is the aim of the project. The project provides training for the student in multidisciplinary yet complementary skills of natural product chemistry, pharmacology and both biochemical and molecular aspects of bone cell biology to achieve the following project goals:

Rotation project – Pharmacokinetic monitoring of acute and multiple doses in disease models.

Year 1 – Measurement of dose dependent effects on biochemical markers of bone turnover.

Years 2 & 3 – Continuation of biochemical marker experiments, progressing translational aspects of the project, but also integrating over-arching biomedical research investigating new mechanisms of action (with Prof Agamemnon Grigoriadis).

This studentship offers a mobility component with our project partners (Australian Institute of Marine Science), and exposure to Intellectual Property and Licensing activities (with Dr Salma Ishaq).

Two representative publications from supervisors:


K65.1 Biomaterials Based on Modulators of Wnt Signalling for Wound Healing

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Project description:
Patients can be affected by a wide variety of wounds, including chronic and acute wounds. With the cost of wound care rising (£9 billion market size), efforts are underway to improve treatment. Genetic studies have implicated Wnt signaling pathway in wound healing and tissue regeneration. However, existing therapeutics have failed to specifically activate the pathway. The proposed project aims to develop novel elastomeric biomaterials that specifically regulate Wnt signaling and to deliver them to the wounded area in a timely controlled manner. Additionally, we aim to activate the stem cells in the area around the wound. This strategy may inhibit scar formation and induce tissue regeneration. If these experiments succeed, these biomaterials will transform the wound’s management and will pave the way for new approaches of treatment.

1st and 2nd years: Purification of the therapeutic proteins Wnt, DKK1 and R-spondin. Developing the hydrogel network to entrap the protein and tethering the proteins to 3 dimensional hydrogel networks and testing their biological effect. The student will learn biomaterials chemistry, protein biochemistry, bioengineering methods and in vitro assays to test protein activity.

3rd- 4th year: The delivery of the different types of biomaterials to the wounded area and observing the healing process in various genetic backgrounds of mice. Furthermore, the student will learn how to bioengineer skin implants for transplantation in the wounded area. During this period the student will learn how to handle animals, animal genetics, histology and advanced imaging techniques.

Two representative publications from supervisors:

K66.1 Defining the role of circulating fibrocytes in the pathogenesis of renal fibrosis; a study of cell-signalling crosstalk

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Project description:
Interstitial fibrosis is the process that leads to kidney failure, regardless of the initiating disease (e.g. diabetes, high blood pressure, kidney transplant rejection), yet there is no drug available to stop this. Understanding which cells are responsible for producing scar tissue and the signalling cascades that control this process will help us to design drugs which can protect patients from the need for dialysis or kidney transplantation. We have evidence that, following injury, circulating cells known as fibrocytes are responsible for damage caused to blood vessels within the kidney. These cells may also migrate into kidney tissue and be responsible for kidney scarring. These cells express a molecule called tissue factor, which triggers the coagulation cascade but also signals through pro-fibrotic signalling pathways such as Ras monomeric GTPases, via PAR-1. We have developed a transgenic strain of mice that overexpress the naturally-occurring human tissue factor pathway inhibitor (TFPI) on CD31+ fibrocytes.

This project aims to
1) Discover what percentage of scar-forming cells are derived from circulating fibrocytes in a mouse model of renal fibrosis (induced by aristolochic acid) (year 1)
2) Compare the degree of fibrosis that develops between the transgenic and wild-type mice to see whether TFPI is anti-fibrotic (year 2)
3) To understand the cross-talk between Ras and PAR-1 signalling to highlight new potential targets for drug discovery (year 3)
4) Test the impact of targeting those signalling molecules that have been newly identified as key in the fibrotic pathway in in-vitro models using CRISPR technology. (year 4).

Two representative publications from supervisors:


K67.1 miR-acles in collecting ducts underlie kidney scarring driven by risk factors

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Project description:
Why do albuminuria, hypertension and excitation of renal nerves cause kidney scarring in chronic kidney disease (CKD)? Our overarching hypothesis is that collecting ducts (CDs) play central roles in these detrimental causal relations.

We found that retinoic acid (RA) activity is physiologically confined to CDs and that albuminuria sequesters RA and significantly reduces RA activity in CDs in CKD mice (Figure 1).

In cultured CD cells, our microarray studies have identified miR29b, miR30e and miR140 as the top three microRNAs (miRs) most suppressed by inhibiting RA biosynthesis and antagonising RA receptors. These 3 miRs directly repress expression of downstream effectors and premier mediators of fibrogenesis, including collagens, Ctgf and Smad3.

We hypothesise that urinary albumin, renal neurotransmitters and mediators of hypertension repress expression of RA/RAR-dependent miR29b, miR30e and miR140, and thus cause kidney fibrosis (Figure 2).

We will address this hypothesis mainly in cultured CD cells and renal fibroblasts. CD-derived urinary exosomes and kidney tissues already collected from CKD patients with and without the concerned risk factors will also be examined.

Year 1: Optimising cellular models (including CD-derived mesenchymal stem cells) for studying the proposed albuminuria-RA/RAR-miR axis; effects of albumin, neurotransmitters and mediators of hypertension on RA/RAR activity in CD cells; pilot studies of urinary exosomes;

Year 2: Stable transfectants of pre-miR, anti-miR or negative control miRs and effects on fibrogenesis; CD-derived urinary exosomes;

Year 3: miR target gene analysis and mechanisms of action in cellular models, including CD-derived mesenchymal stem cells.

Year 4: Animal models to validate in vitro findings and to explore intervening new strategies.

Two representative publications from supervisors:


K68.1 Genetic epidemiology of age-related hearing impairment, analysis of pathological processes and identification of therapeutic targets

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Project description:
Age-related hearing impairment (ARHI) is a significant health and social problem, impacting significantly on quality of life, and is likely caused by multiple environmental and genetic factors. Genetic influence is strong, with estimated heritability as high as 70%, but few genetic variants influencing ARHI have been reliably determined. As part of the HEAR-UK consortium, we are starting to analyse data from the UKBiobank including a measure of auditory function (Speech-in-Noise test) and genotyping data, to carry out a Genome-Wide Association Study (GWAS). This study is substantially larger (n=150,000) than anything before and is highly likely to find and validate (through replication) many more genetic variants associated with ARHI. Results of the GWAS will be used to explore the function of the genes identified, firstly studying normal distribution of expression in the cochlea, followed by analysis of auditory function in at least one mouse mutant with one of the candidate genes inactivated.

Objectives
1. to identify new genes associated with ARHI by performing by far the largest genetic association study to date (Williams, year 1)
2. to validate the genes identified using suitable mouse models (Steel, years 2-3)
3. to define the regulatory pathways involved in hearing impairment to gain mechanistic insight into the pathological processes and to identify therapeutic targets (Steel & Williams, years 2-3)

Skills training: statistics; GWA analysis; mouse genetics & genotyping; immunocytochemistry; qRTPCR; Auditory Brainstem Response measurements in mouse; ultrastructural and/or confocal analysis of the cochlea; pathway analysis.

Two representative publications from supervisors:

K69.1 Molecular mechanisms of resistance to therapy in lymphoma

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Co-Supervisor 2: Dr Paul Fields
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Project description:
Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in adults. The outcome of DLBCL therapy has greatly improved since the introduction of rituximab (anti-CD20 antibody) into the standard chemotherapy regime using cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). Despite the success, a significant proportion of patients ~30-40% will be refractory to R-CHOP or relapse at a later date. Here we propose: 1. (Years 1-2) to identify molecular alterations present in paired presentation-relapsed and refractory R-CHOP-treated DLBCL patients; 2. (Years 1-2) use high-end mouse genetics to establish a model of DLBCL where standard R-CHOP leads to relapsed and/or refractory disease, and to investigate the molecular alterations responsible for that outcome; 3. (Years 3-4) perform inter-species oncogenomic comparisons to identify recurrent genetic lesions in human and mouse either present in the same gene or within the same signalling/genetic pathway, and test their ability to enhance a refractory or relapse disease response to R-CHOP using the DLBCL mouse model system. The project involves training skills in high throughput sequencing including whole exome-sequencing; bioinformatic analysis of high data content; pathological characterisations of affected organs using immunohistochemistry and fluorescence in-situ hybridization; analysis of tumour cells by multicolor flow-cytometry; whole mouse body imaging techniques including PET and ultrasounds; models of disease. Translationally these studies aim to identify mechanisms of resistance to R-CHOP, with possible novel biomarker discovery, which could be used to risk-stratify patients to the appropriate intensity of first line of treatment, and to inform medical decision making required for more effective alternative therapies.

Two representative publications from supervisors:
*Equal Contribution and Correspondence

K70.1 Genome-wide and epigenetic screen in drug metabolising (ADME) genes in twins

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Project description:

Scientific basis:
This proposal will examine a genome-wide screen of relevant gene expression and DNA methylation quantitative trait loci (eQTL and meQTL) across multiple tissues in drug metabolising ADME (absorption, distribution, metabolism, and excretion) genes in the TwinsUK cohort. We will use genetic analysis to detect relationships between genetic polymorphisms, epigenetic and gene expression, adjusting for confounders, focusing on enrichment of signals at ADME genes. Heritability of metabolic signatures, associated with genetic variation in ADME will also be explored. TwinsUK is the biggest UK adult twin registry (12,000) to study genetic and environmental etiology of age related complex traits, drug metabolism and diseases.

Skills training
1. Translational pharmacogenetics
   Study design overview: Translational pharmacogenetics; large datasets; data resources & methods for handling bias and confounding - including analytical models; GWAS; eQTL; meQTL; meta-analysis; evidence synthesis.

2. Use and application of epigenetics
   How epigenetic change (heritable changes in gene expression) can affect phenotype via DNA methylation, histone modification and micro regulation (miRNA) processes. Explore role of factors such as age, environment and disease on epigenetic and gene expression profiles at ADME genes CYP3A4/CYP1A2/CYP1B1/MDR.

Objectives
Yr 1: Explore GWAS for eQTL and meQTL in major drug metabolising (ADME) genes focusing on targeted analyses of specific genes, e.g. genes affecting drug metabolism through renal and hepatic pathways.
Yr 2: Determine heritability and genetic basis (mQTLs) of metabolomics profiles in ADME genes.
Yr 3: Examine links across genetic, epigenetic, expression, and metabolomic signatures at identified ADME QTLs across multiple layers of omic data.

Two representative publications:
1. Epigenetics of discordant monozygotic twins: implications for disease
   Castillo-Fernandez, J. E., Spector, T. D. & Bell, J. T. 31 Jul 2014 In : Genome medicine. 6, 7, 60
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