University of Kyoto

John Mung Scholarship

Radcliffe Department of Medicine
University of Oxford

www.rdm.ox.ac.uk

6 month research projects

Radcliffe Department of Medicine

1	Hydrogen Sulfide as a signalling molecule in the cross-talk between perivascular fat and the vascular wall in humans
Supervisor:	**Dr Charalambos Antoniades**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/charalambos-antoniades

Project Description:

Background: Recent evidence suggests that hydrogen sulfide (H2S), a gasotransmitter produced in the adipose tissue, is a key mediator for the vasorelaxing properties of perivascular adipose tissue (PVAT). It penetrates the vascular wall leading to activation of the ATP sensitive K+-channels in the vascular smooth muscle cells, inducing vasorelaxation (1). H2S also activates PI3Kinase/Akt signalling in the vascular cells and this affects the phosphorylation/activation of enzymes like endothelial nitric oxide synthase(2).

In our group we use ex vivo models of human adipose tissue(3), vessels(4,5) and myocardium(6), to study the mechanisms governing the cross-talk between adipose tissue and the vascular wall. We have developed the Oxford CABG Bioresource (OCB) (7), one of the world's most extensively phenotyped cohorts of patients with advanced atherosclerosis, designed to explore the molecular signaling between adipose tissue and the cardiovascular system, using non-invasive cardiovascular imaging, ex vivo models of human vessels/myocardium/adipose tissue and cell culture techniques on primary cells isolated from these patients. The current project will use resources from the OCB project.

Aim: The aim of this project is to characterize the paracrine effects of H2S released from human adipocytes/preadipocytes on vascular redox signalling in human atherosclerosis.

Brief description: The project will use human vessels, adipose tissue and existing data from OCB, to search for the interactions between H2S production in human PVAT and vascular redox signalling . The use of OCB will allow us to understand the importance of individual patient characteristics (e.g. diabetes, obesity, hypertension etc) as modifiers of either the production of H2S in the human adipose tissue or the responsiveness of the human vessels to it. By using cell culture methodology, the student will explore the mechanisms by which H2S produced by each individual enzymatic source in the human adipocytes and pre-adipocytes, affects important aspects of human vascular biology, focusing on its effects on redox signalling in human vascular smooth muscle cells. The experiments will be carried out in primary cells isolated from patients undergoing cardiac surgery, and the results will be validated in ex vivo models of human vessels and adipose tissue. The clinical significance of any findings from the cell culture/ex vivo models will then be tested the context of the OCB cohort.

Training Opportunities:

The student will be trained in: Primary cells isolation and culture; Cells migration and proliferation assays; Cells transfection (transient and stable); Fluorescence microscopy; Co-culture of primary cells; ROS detection (lucigening chemiluminescence, DHE quantification by HPLC); DNA/RNA extraction and purification; PCRs, RT-PCRs and qPCRs; Quantification of H2S by using the amperometric method and methylene blue assay

References:

1. Zhao W, ey al; *The EMBO journal.* 2001;20(21):6008-6016.
2. Coletta C, et al; *PNAS* 2012;109(23):9161-9166.
3. Antonopoulos AS, et al; ATVB 2014 (in press).
4. Antoniades C, et al; *Circulation.* 2011;124(17):1860-1870.
5. Antoniades C, et al; *Circulation.* 2011;124(3):335-345.
6. Antoniades C, et al; *J Am Coll Cardiol.* 2012;59(1):60-70.
7. Margaritis M, et al; *Circulation.* 2013;127(22):2209-2221.

2	hES and hIPS derived cardiomyocyte models of Inherited Heart Disease

Supervisor:	**Dr Matthew Daniels**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/matthew-daniels

Project Description:

I started a small group in Dec 2012 so our work is currently unpublished. We are based on the John Radcliffe site.

We recruit patients from the Universities large Inherited heart disease clinic, and reprogram these to pluripotency using the OSKM factors delivered by a single Sendai virus1 (collaboration Dr Nakanishi, AIST, Tsukuba, Japan). We are then able to produce large numbers of cardiomyocytes from this material2 and use these as a substrate to try and determine the mechanism of disease using a combination of live cell imaging methods, genetically encoded reporter systems3 (collaboration Prof Take Nagai, SANKEN, Osaka, Japan) and viral delivery systems.

We could offer a range of six month projects depending on the need/experience of the particular candidate – feasible options for this timescale would include

- Patient iPS generation & characterisation – for example where we have access to material that the Kyoto institution does not
- hES/iPS maintainence & cardiomyocyte differentiation and characterisation – to enable the Kyoto institution to generate an indefinitely renewable source of cardiomyocytes
- Genetically encoded reporter development/delivery for application to cardiomyocytes

Although typically we would expect these projects to appeal to biological/biomedical candidates we would be prepare to train candidates from a materials or computer science/mathematics background accepting that the familiarity with biological methods may be less developed than a life science trainee.

Training Opportunities:

Human stem cell growth/maintainance; Cardiomyocyte differentiation; Molecular cloning; live cell imaging; viral production; data analysis

References:

1 - Development of defective and persistent **Sendai** virus vector: a unique gene delivery/expression system ideal for cell **reprogramming**. (Nishimura et al, JBC, 2011, 286(6):4760-71)

2 - Robust **cardiomyocyte differentiation** from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. (Lian et al, PNAS, 2012, 109, e1848-57)

3 - An expanded palette of genetically encoded Ca^{2+} indicators (Zhao et al, Science, 2011, 333, 1888-91)

3	Deciphering microRNA regulatory networks using a novel genome engineering research pipeline

Supervisor:	**Dr Tudor A. Fulga**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/tudor-fulga

Project Description:

Every cell within our body carries the same genetic information, yet following iterative developmental transitions hundreds of morphologically and functionally distinct cell types are generated. At the foundation of this fascinating cellular diversification, lies a milieu of finely orchestrated and sophisticated regulatory programmes, which act to turn on or off thousands of genes (~20,000 in humans) with minute spatial and temporal precision. Errors in these programmes can give rise to developmental defects and many human diseases including cancer. One such essential regulatory layer is provided by microRNAs, which bind and tune the expression of numerous cellular RNAs via defined "microRNA response elements" (MREs). Although tremendous progress has been made towards understanding the biological role of microRNAs, their broad impact on cellular target genes remains elusive. Addressing this question requires a systems level approach, which hitherto had been technically unfeasible. To elucidate this fundamental facet of microRNA biology, we have developed a powerful experimental platform that uses novel genome engineering technologies (CRISPR/Cas9) to interfere with the activity of MREs in the context of an intact biological system. We are currently extending these studies to systematically characterise every link within one specific microRNA cellular network. This will enable us for the first time to understand, predict and assess the impact of microRNAs in the context of a complete gene regulatory network. Our long-term goal is to elucidate the entire landscape of human microRNA networks in normal versus disease states, applying this knowledge to the design of microRNA-based therapeutic strategies

Training Opportunities:

This project is carried out at University of Oxford's Weatherall Institute of Molecular Medicine, in a highly dynamic and competitive environment. Interdisciplinary by design, the project will involve a broad range of cutting edge technologies for studying non-coding RNAs including state of the art techniques for target identification (bioinformatics, Ago-HITS-CLIP), high throughput sequencing (MiSeq), advanced molecular biology, genome engineering (TALEN/CRISPR), RNA biochemistry, and computational biology. In addition, the student will be trained to develop writing and presentation skills.

References:

Bassett, A., Azzam, G., Wheatley, L., Tibbit, C., Stanger, N., Ponting, C.P., Liu, J.L., Sauka-Spengler, T., Fulga, T.A. (2014) *Understanding functional miRNA-target interactions in vivo by site-specific genome engineering.* **Nature Communications**. *In press.*

Loya C.M., Lu C.S., Van Vactor D., and Fulga T.A. (2009) *Transgenic microRNA inhibition with spatiotemporal specificity in intact organisms* **Nature Methods**, 6(12):897-903.

Fulga, T.A, Elson-Schwab, I., Khurana, V., Steinhilb, M.L., Spires, T.L., Hyman, B.T. and Feany, M.B. (2006) *Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration in vivo.* **Nature Cell Biology**, 9(2):139-48.

4	The Role of Stretch Signalling in Hypertrophic Cardiomyopathy
Supervisor:	**Dr Katja Gehmlich**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/katja-gehmlich

Project Description:

Hypertrophic cardiomyopathy (HCM) is a common genetic order of the heart, affecting 1 in 500 people. While the majority of cases are caused by mutations in sarcomeric genes, a small subset is caused by mutation in genes coding for cardiac signalling proteins. The latter is poorly understood so far.

This project will look into the contribution of aberrant stretch signalling to the pathogenesis of HCM. In more detail, HCM-causing mutations in CSRP3 (coding for Muscle LIM Protein) and ANKRD1 will be investigated and perturbations of signalling pathways caused by the presence of the mutations will be investigated. The mutations will be introduced into rodent cardiomyocytes by adenoviral transduction and cell cultures will be subjected to mechanical stress.

In parallel tissue from a mouse model mimicking a human CSRP3 mutation will be investigated for alterations in cardiac signalling pathways and cardiac cells will be isolated from these mice and subjected to mechanical stress.

The project will help to gain better insight into patho-mechanisms of HCM, potentially providing the basis for novel therapeutic avenues.

Training Opportunities:

Molecular and biochemical laboratory techniques (e.g. quantitative PCR, Western blotting). Work with primary cells (neonatal rodent cardiomyocytes), adenoviral gene delivery and analysis of tissue from genetically modified mice. In vitro stretch of cell cultures (Flex Cell system).

Experimental design, data analysis and interpretation.

References:

Impact of ANKRD1 mutations associated with hypertrophic cardiomyopathy on contraction parameters of engineered heart tissue.
Crocini C, Arimura T, Reischmann S, Eder A, Braren I, Hansen A, Eschenhagen T, Kimura A, Carrier L.
Basic Res Cardiol. 2013;108(3):349. doi: 10.1007/s00395-013-0349-x. PMID: 23572067

Beyond the sarcomere: CSRP3 mutations cause hypertrophic cardiomyopathy.
Geier C, Gehmlich K et al. Hum Mol Genet. 2008 Sep 15;17(18):2753-65. doi: 10.1093/hmg/ddn160.
PMID: 18505755

Back to square one: what do we know about the functions of muscle LIM protein in the heart?
Gehmlich K, Geier C, Milting H, Fürst D, Ehler E.
J Muscle Res Cell Motil. 2008;29(6-8):155-8. doi: 10.1007/s10974-008-9159-4. PMID: 19115046

5	Development of Gene Therapy Vectors for Cystic Fibrosis

Supervisor:	**Dr Deborah Gill**
Website:	http://www.genemedresearch.ox.ac.uk/

Project Description:

Interest in gene therapeutic strategies has been reinvigorated since the recent approval of the first European gene therapy product. We have many years of expertise in the development of novel viral and non-viral gene transfer vectors, several of which are being evaluated in clinical trials. Specifically, we are developing gene therapies for lung diseases, including treatment of the monogenic lethal disease Cystic Fibrosis (CF), which aims to replace the defective epithelial chloride channel (CFTR) in the airways.

Transgenic CF pig and CF ferret models have been generated that develop CF-like lung pathologies. In order to assess our gene therapy strategies in these animal models, new vectors expressing pig and ferret cDNA are required. This project involves the construction of new gene transfer vectors expressing CFTR from pig and ferret. To confirm that these vectors express functional CFTR channels, a new cell-based assay of CFTR channel function will be developed. The assay will use an iodide-sensitive electrode to measure iodide efflux as a surrogate for the movement of chloride ions through the CFTR channel. The assay will be established using human CFTR permanently expressed in stable cell lines and will be used to screen gene transfer vectors based on plasmid DNA and lentiviral vectors. In addition, this assay will form part of the collection of GMP release assays required to assess the quality of our lentiviral vector manufacturing processes, prior to use of gene therapy products in the clinic.

Training Opportunities:

Our research group is based in the John Radcliffe Hospital and our research is fully translational from the development of new viral vectors through testing, toxicity studies, manufacturing and clinical trials; an excellent opportunity to learn about research from a translational viewpoint.

Techniques involved in this project could include: Gene transfer in cell culture; quantitative TaqMan RT-PCR; Western blotting; vector construction, sequencing & molecular biology; and viral manufacture.

Students can also attend a wide-range of courses, workshops and seminars held by the University.

References:

http://www.ncbi.nlm.nih.gov/pubmed/25015239

http://www.ncbi.nlm.nih.gov/pubmed/24865497

http://www.ncbi.nlm.nih.gov/pubmed/23525080

http://www.ncbi.nlm.nih.gov/pubmed/22955314

http://www.ncbi.nlm.nih.gov/pubmed/18438402

6	**Prediction of long term whole organ pancreas transplant survival**
Supervisor:	**Professor Stephen Gough**
Website:	http://www.ocdem.ox.ac.uk/grant-holders/researcher/stephen-gough

Project Description:

Lifelong exogenous insulin replacement is the mainstay of treatment for the vast majority of people with type 1 diabetes. Some people however with long term complications including end stage renal disease and difficult to treat diabetes undergo beta cell replacement therapy in the form of either whole organ pancreas transplantation or isolated islet cell transplantation. Whole organ pancreas transplantation can result in improved mortality rates and excellent long-term graft function and independence from insulin injections. Unfortunately individual grafts do fail and it is difficult to predict these ahead of a return to a renewed requirement for insulin injections.

The aims of this programme of work are to identify early predictors of long term graft survival/failure. Individual projects will focus on potential predictors of long term graft function by analysing pre-transplant clinical profiles, polymorphism of donor and recipient DNA and detailed static and dynamic physiological measurements (utilising frequently sampled oral glucose tolerance tests, glucose clamps, incretin hormone profiling and mathematical modelling of beta cell function). The identification of predictive markers of graft function will facilitate the targeting of specific therapies aimed at improving long term graft survival and insulin independence for people with type 1 diabetes.

7	Understanding vascular phenotype of mother and child following pre-eclampsia

Supervisor:	**Professor Paul Leeson (PI) and Dr Grace Yu (Post-doc)**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/paul-leeson

Project Description:

A pregnancy complicated by pre-eclampsia identifies both a mother and child predisposed to develop cardiovascular risk factors and diseases such as hypertension, stroke and myocardial infarction (Davis et al Pediatrics 2012). Therefore, characterisation of biological pathways common to both pre-eclampsia and cardiovascular disease offer the opportunity to develop novel insights into both conditions and lead to development of new preventive interventions.

Over the last few years our group has identified specific changes in vascular biology in the offspring of pregnancies complicated by pre-eclampsia (Lazdam et al Hypertension 2010) and that certain subgroups of mothers and children, namely those born to early-onset disease, have unique differences in their disease process both during pregnancy and throughout later life (Lazdam et al Hypertension 2012).

This project will make use of a tissue bioresource of umbilical-derived primary vascular cells with linked clinical physiological and medical record data to dissect out mechanisms underlying vascular dysfunction in the offspring (Davis et al Clin Sci 2012).

Training Opportunities:

This project can be used to gain experience in cell biology techniques including; tissue culture, cell transfection, FACS, RNA interference, and immunofluorescence microscopy. There will also be the opportunity for bioinformatics analysis. As there is ongoing collection of clinical samples with linked physiological data there will be opportunities for interested, and appropriately experienced, individuals to combine laboratory research with 'bedside' data collection.

References:

Davis EF, Newton L, Lewandowski AJ, Lazdam M, Kelly BA, Kyriakou T, Leeson P. 2012. Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies. Clin. Sci., 123 (2), pp. 53-72.

8	Construction of Chimeric Antigen Receptor (CAR) engineered T-cells

Supervisor:	**Dr Demin Li, Professor Alison Banham**
Website:	http://www.ndcls.ox.ac.uk/llr-molecular-haematology-unit

Project Description:

Novel breakthroughs using immune checkpoint inhibitory antibodies and engineered T-cells have fuelled a global explosion of interest in targeting the immune system to successfully treat previously incurable cancers. The host lab comprises a diverse team of experienced researchers developing novel therapeutic monoclonal antibodies for the treatment of cancer. One group of targets are peptides from intracellular tumour restricted proteins presented on the cancer cell surface by major histocompatibility complex (MHC) molecules. T-cell receptor mimic (TCRm) antibodies against these targets have been developed using hybridoma technology, enabling the targeting of intracellular proteins; which far outnumber the traditional antibody biomarker targets available on the cell surface. TCRm antibodies can be combined with chemotherapy, developed as antibody-drug conjugates, or used as the targeting agent for engineered T cells. This project will use gene-cloning technology to generate novel chimeric antigen receptor (CAR) expression constructs expressing our lead TCRm antibody (other antibody targets are also available). Stable T-cell lines and primary cells will be generated by lentiviral transduction with these CAR constructs and tested for their tumour specific binding and functional efficacy against cancer cells. These CARs provide the starting point for the development of novel TCRm/CAR cancer therapeutics.

Training Opportunities:

This project will provide training in molecular biology techniques, such as gene cloning, real-time PCR, lentiviral gene delivery; immunology techniques such as flow cytometry and cell biology techniques such as culture of cell lines and primary T cells, cytotoxicity, cell proliferation, apoptosis and luciferase assays. Students are eligible to attend the wide-range of courses held by the University, and will experience state of the art seminars given by internal and visiting scientists.

References:

1. Massimo Masiero, Filipa Costa Simões, Hee Dong Han, Cameron Snell, Tessa Peterkin, Esther Bridges, Lingegowda S. Mangala, Sherry Yen-Yao Wu, Sunila Pradeep, Demin Li, Cheng Han, Heather Dalton, Gabriel Lopez-Berestein, Jurriaan B. Tuynman, Neil Mortensen, Ji-Liang Li, Roger Patient, Anil K. Sood, Alison H. Banham, Adrian L. Harris, Francesca M. Buffa. A core human primary tumor angiogenesis signature identifies ELTD1, an endothelial orphan receptor regulating angiogenesis. Cancer Cell 2013; 24(2): 229-41. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3743050/
2. Kershaw, M.H., Westwood, J.A., and Darcy, P.K. (2013). Gene-engineered T cells for cancer therapy. Nature reviews Cancer 13, 525-541. http://www.nature.com/nrc/journal/v13/n8/full/nrc3565.html
3. Rony Dahan and Yoram Reiter (2012) T-cell-receptor-like antibodies – generation, function and applications. Expert Rev. Mol. Med. Vol. 14, e6, February 2012, doi:10.1017/erm.2012.2 http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=8498934&fileId=S1462399412000026

9	Relationships between T2D and fasting glucose GWAS and exome sequence results and tissue expression patterns

Supervisor:	**Professor Mark McCarthy**
Website:	http://www.ocdem.ox.ac.uk/grant-holders/researcher/mark-mccarthy

Project Description:

The work of the McCarthy group, based at OCDEM and at the Wellcome Trust Centre for Human Genetics, is focused around using human genetics as a tool for understanding the mechanisms responsible for type 2 diabetes pathogenesis. We have led efforts to generate large global data sets based around genome wide association (eg the DIAGRAM consortia) and whole genome and exome sequence data (GoT2D and T2DGENES consortia). The former has led to identification of around 70 loci robustly associated with type 2 diabetes pathogenesis (though the specific transcripts involved are not always clear), and the latter is revealing novel coding variant signals. In this project, we are interested in the incomplete overlap in the genetic signals discovered for type 2 diabetes, and those shown to be influencing fasting glucose levels in normoglycemic individuals: some loci have strong effects on both, but others seem to influence one or the other. This project will examine the transcripts mapping within GWAS loci, and those contributing to signals in exome sequence and exome chip data sets, and relate differential phenotypic effects, to patterns of tissue specific expression as defined in both public and proprietary data sets. The objective is to understand whether differences in tissue effects might contribute to the divergence of physiological and pathological effects.

Training Opportunities:

The project will provide expertise in bioinformatics and computational analysis relevant to diabetes, as well as exposure to large-scale genetic data sets. Some experience of computing (especially PERL, but also R, C, UNIX) would be desirable for those interested in this project.

References:

http://www.ncbi.nlm.nih.gov/pubmed/22885922

http://www.ncbi.nlm.nih.gov/pubmed/22885924

10	Semantic analysis of published abstracts to reveal pathways implicated in type 2 diabetes pathogenesis
Supervisor:	**Professor Mark McCarthy**
Website:	http://www.ocdem.ox.ac.uk/grant-holders/researcher/mark-mccarthy

Project Description:

The work of the McCarthy group, based at OCDEM and at the Wellcome Trust Centre for Human Genetics, is focused around using human genetics as a tool for understanding the mechanisms responsible for type 2 diabetes pathogenesis. We have led efforts to generate large global data sets based around genome wide association (eg the DIAGRAM consortia) and whole genome and exome sequence data (GoT2D and T2DGENES consortia). The former has led to identification of around 70 loci robustly associated with type 2 diabetes pathogenesis (though the specific transcripts involved are not always clear), and the latter is revealing novel coding variant signals. In this project, the plan will be to interrogate this growing list of genes implicated in type 2 diabetes risk using semantic relationships defined within PubMed abstracts, using tools such as GRAIL. The aim will be to quantify the evidence for aggregation of T2D-related genes within the published literature, and to determine whether there is additional evidence in support of that aggregation from other data sources (eg protein-protein interaction, or co-expression data).

Training Opportunities:

The project will provide expertise in bioinformatics and computational analysis relevant to diabetes, as well as exposure to large-scale genetic data sets. Some experience of computing (especially PERL, but also R, C, UNIX) would be desirable for those interested in this project.

References:

http://www.ncbi.nlm.nih.gov/pubmed/19557189

http://www.ncbi.nlm.nih.gov/pubmed/22885922

11	Exploring the role of histone 3 lysine 27 demethylases in gene regulation and acute leukaemia growth

Supervisor:	**Dr Thomas Milne**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/thomas-milne

Project Description:

There has been much progress in treating human cancers, especially leukaemias, but many remain resistant to treatment. A potentially exciting approach is the development of small molecule inhibitors that specifically target aberrant processes in cancer cells but leave normal cells unharmed. In order to be successful, such an approach requires highly detailed information about normal and aberrant cellular processes on the molecular level.

In cooperation with genetic mutations, epigenetic aberrations are a major driving force in human cancers. Epigenetic changes are generally defined as heritable changes in gene expression that do not alter the underlying DNA sequence. One way epigenetic information can be stored is by covalently labelling specialized proteins called histones with “marks” such as methylation. Histone 3 lysine 27 trimethylation (H3K27Me3) is a mark that recruits a “reader” complex called the polycomb group and causes gene repression. Removing the H3K27Me3 mark is controlled by the demethylases UTX and JMJD3 and causes gene activation.

This project focuses on answering the following question: Do either of the two known H3K27Me3 demethylases, UTX or JMJD3, help maintain leukaemic cell growth through the demethylation of H3K27Me3 and the activation of genes?. The approach will be to induce in vitro gene knockdowns/knockouts of these proteins in leukemia cells, measure the effect on leukemia growth, and perform genome wide ChIp-sequencing and RNA-sequencing studies to determine the effects on gene expression.

Training Opportunities:

Work with leukaemia cells in tissue culture, genome editing knockout approaches, optimizing protocols for ChIP-sequencing and an introduction to bioinformatic analyses.

References:

Two key papers:
http://www.sciencedirect.com/science/article/pii/S221112471300003X
http://www.sciencedirect.com/science/article/pii/S1097276510003734

Google scholar link:
http://scholar.google.co.uk/citations?hl=en&user=JFxz2vQAAAAJ&view_op=list_works

12	Sodium channels, chronic pain and insulin secretion

Supervisor:	**Professor Patrik Rorsman**
Website:	http://www.ocdem.ox.ac.uk/grant-holders/researcher/patrik-rorsman

Project Description:

Insulin-secreting mouse pancreatic beta-cells express voltage-dependent sodium channels. Single-cell PCR has revealed that these channels consist of Nav1.7 alpha subunits. Intriguingly, the sodium current in beta-cells undergo voltage dependent inactivation at unphysiologically negative membrane potential; half-maximal inactivation is observed at -105 mV and the current is completely inactivated at -80 mV (the most negative membrane potential of the beta-cell observed physiologically). Although the sodium currents are very large (10-fold bigger than the calcium current involved in insulin secretion) i, they are not necessary for beta-cell electrical activity/insulin secretion. The role of beta-cell sodium channels in beta-cells therefore remains an enigma.

It is of interest that exactly the same sodium channels are expressed in nociceptive (pain-sensing) neurones. A selective blocker of Nav1.7 channels would be a very useful addition to treat chronic pain and several pharmaceutical industries currently attempt to develop such blockers. Surprisingly, the sodium channels in mouse neurones inactivate at ~40 mV more positive voltages than in beta-cells. This is not because beta-cells and neurones express distinct splice variants of the channel. Collectively, these observations suggest that beta-cells contain a protein or factor that shifts the inactivation of Nav1.7 sodium channels into the unphysiological range of membrane potentials.

We will identify this factor by expressing Nav1.7 channels in HEK cells and the 'rebuild' the beta-cell by expressing key proteins present in beta-cells (but not in neurones). For example, we will test the impact of expressing the sulphonylurea receptor SUR1 (that is one of two subunits of the ATP-regulated potassium channel). We will also explore the impact of metabolic factors by exposing recombinant Nav1.7 channels in excised HEK cell membranes to a beta-cell cytosolic extract. Although the project deals with the biophysics of sodium channels, there is a potential translational component. If neuronal Nav1.7 channels could be made to inactivate like their beta-cell counterparts, then this might represent a novel strategy for producing pain relief in patients with chronic pain.

Finally, it is of interest to elucidate why the beta-cell express Nav1.7 channels at such a high density. Possibly, they subserve functions other than ion conduction and action potential generation?

Training Opportunities:

The project will expose the student to a breadth of state-of-the-art cell electrophysiological techniques, imaging, cell culture and expression of recombinant proteins.

13	Defining 'super enhancer' signature for neural crest fate

Supervisor:	**Dr Tatjana Sauka-Spengler**
Website:	http://www.rdm.ox.ac.uk/principal-investigators/researcher/tatjana-sauka-spengler

Project Description:

The neural crest (NC) is a multipotent stem cell-like embryonic population that gives rise to a wide range of derivatives in the vertebrate body, including sensory neurons and glia, cartilage, bone and connective elements of craniofacial skeleton and the vast majority of body's pigmentation[1,2]. Neural crest progenitors arise at the end of gastrulation and as neurulation proceeds, they are found within the dorsal neural tube; they then undergo an epithelial-to-mesenchymal transition, changing their morphology and adhesion properties, before migrating throughout the embryo. Our laboratory focuses upon deciphering the gene regulatory circuitry that orchestrates cellular processes in the premigratory NC. Those involve mechanisms of NC cell specification and maintenance of their multipotency. To perform global analyses of the NC cis-regulatory circuits we have generated epigenomic and transcriptional maps of cranial NC cells from chicken embryos, identifying tissue-specific cis-regulatory elements genome-wide. The project proposed here will involve characterising function of these elements, focusing in particular on the discernable 'super enhancer' signatures that may control NC cell identity in vivo. We will use:

(i) genome engineering methods to apply DNA-programmable fusion proteins and deliver chromatin modifying activity to putative enhancers[3], as well as to delete functional binding domains and assay their functional relevance.

(ii) multiplex reporter assays to define spatio-temporal patterns of enhancer activity.

(iii) Capture-C approach[4] recently adapted to small cell numbers to analyse enhancer conformation and long distance interactions with relevant NC loci.

Training Opportunities:

Small cell number epigenomic profiling, genome-engineering (Crispr/Cas9 system, Cas9-LSD1, etc.), Capture-C, fluorescent reporter assays, confocal microscopy.

References:

1. Sauka-Spengler, T. and M. Bronner-Fraser, *A gene regulatory network orchestrates neural crest formation.* Nature reviews. Molecular cell biology, 2008. **9**(7): p. 557-68.
2. Simões-Costa M, Tan-Cabugao J, Antoshechkin I, Sauka-Spengler T, Bronner ME (2014). *Transcriptome analysis reveals novel players in the cranial neural crest gene regulatory network.* Genome Res., 2014 **24**(2):281-90.
3. Mendenhall, E.M., K.E. Williamson, D. Reyon, J.Y. Zou, O. Ram, J.K. Joung, and B.E. Bernstein, *Locus-specific editing of histone modifications at endogenous enhancers.* Nat Biotechnol, 2013. **31**(12): p. 1133-6.
4. Hughes, J.R., et al., *Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment.* Nat Genet, 2014. **46**(2): p. 205-12.

14	Regulating Human Haematopoietic Stem Cell Self-Renewal for Genome Editing

Supervisor:	**Professor Suzanne Watt**
Website:	www.stemcells.ox.ac.uk; www. rdm.ox.ac.uk; www.ndcls.ox.ac.uk

Project Description:

Our research focuses on improving treatment outcomes for severely ill patients suffering from blood diseases by understanding basic mechanisms and applying this to graft engineering. Haematopoietic stem cells (HSCs) give rise to all blood lineages and are critical to life. However, HSC disorders exist, with haematological malignancies representing a significant cause of mortality and the inherited haemoglobin disorders being the most common monogenic diseases worldwide. Such diseases can potentially be cured by haematopoietic stem cell transplantation (HSCT), the most successful regenerative medicine therapy to date with the year 2013 marking the one millionth HSCT worldwide. Combining stem cell and novel targeted genome editing technologies provides powerful tools for correcting inherited gene disorders in autologous HSCs before transplanting them into the affected individual to correct the disease. Controlling the self-renewal and differentiation in HSCs ex vivo prior to transplantation is critical to their success in correcting a genetic defect, requiring an understanding of the transcriptional regulatory networks and cues that control the balance between human HSC self-renewal and commitment. This project aims to examine specific regulatory networks in HSC/HPC subsets at the single cell level performed using Dynamic Array integrated fluidics chips on the BioMark HD platform combined with specific environmental cues from the bone marrow niche affect their fate and ahead of gene editing using CRISPR technology. In this way, it will be possible to regulate the balance between human HSC self-renewal and their lineage commitment and to optimise human HSCs for gene editing.

Training Opportunities:

The student will have access to a wide-range of different sources of training, both in advanced scientific techniques and transferrable skills. These will include:

i) hands-on training in stem/progenitor cell culture, MACS and FACS isolation and analysis of HSC/HPC subsets, molecular techniques (e.g. RNA extraction, single cell high throughput gene analysis), confocal microscopy and advanced imaging;
ii) scientific skills training through the Methods and Techniques course run by the Weatherall Institute of Molecular Medicine, ensuring that students have the opportunity to build a broad-based understanding of differing research techniques;
iii) generic skills training, e.g. General Health & Safety, Information Governance, Quality Management Systems, Medical and Cryogenic Gases Use. Personal Effectiveness, Research Governance.

References :

1) Moignard V et al. Characterization of transcriptional networks in blood stem and progenitor cells using high-throughput single-cell gene expression analysis. Nat Cell Biol. 2013;15(4):363-72.
2) Pepperell EE, Watt SM. A novel application for a 3-dimensional timelapse assay that distinguishes chemotactic from chemokinetic responses of hematopoietic CD133(+) stem/progenitor cells. Stem Cell Res. 2013 Sep;11(2):707-20.
3) Carpenter L et al. Human induced pluripotent stem cells are capable of B-cell lymphopoiesis. Blood. 2011;117(15):4008-11.

15	Stem Cells for Tissue Repair

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Project Description:

Generating a blood supply from stem cells is fundamental to most tissue repair, while its dysregulation can contribute to serious disease. Indeed, vascular diseases (cardiovascular, stroke etc.) are leading causes of morbidity and mortality worldwide, but less known is the fact that in the UK and USA alone chronic skin wounds affect over 6.7 million patients and many more individuals worldwide. This burden is growing rapidly with an aging population and a sharp rise in diabetes and obesity worldwide. Future therapies will use better defined stem cells and regenerative medicine products, and more personalised and tissue specific approaches. Our objectives are therefore i) to understand the mechanisms of blood vessel formation in tissues; and ii) to develop clinical grade stem/progenitor cells/biologics to support blood vessel formation (e.g. in the heart and skin). However, the co-ordination of stem cell activities within organs is still not well understood. This project therefore aims to treat non-healing wounds using stem cells, by examining the mechanisms at the cellular and molecular levels whereby mesenchymal stem/progenitor cells with different potentialities or from different sources provide a specialised niche that promotes blood vessel formation by endothelial stem/progenitor cells and to test their efficacy using in vitro and/or in vivo models. In this way, it should be possible to produce a suitable tissue engineered graft of good textural durability, associated with minimal scarring and minimal contracture, that will repair non-healing skin wounds.

Training Opportunities:

The student will have access to a wide-range of different sources of training, both in advanced scientific techniques and transferrable skills. These will include:

i) hands-on training in stem/progenitor cell culture, MACS and FACS isolation and analysis of HSC/HPC subsets, molecular techniques (e.g. RNA extraction, single cell high throughput gene analysis), confocal microscopy and advanced imaging;

ii) scientific skills training through the Methods and Techniques course run by the Weatherall Institute of Molecular Medicine, ensuring that students have the opportunity to build a broad-based understanding of differing research techniques;

iii) generic skills training, e.g. General Health & Safety, Information Governance, Quality Management Systems, Medical and Cryogenic Gases Use. Personal Effectiveness, Research Governance.

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1) Papadakis M et al. Tsc1 (hamartin) confers neuroprotection against ischemia by inducing autophagy. Nat Med. 2013 Mar;19(3):351-7.

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