NDM Summer Internship Projects 2018

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The role of HIF in proliferation and cancer: carotid body physiology/pathology as a paradigm for pseudohypoxic cancers

Hypoxia is common to many cancers, as the oxygen needs of proliferating tumour cells cannot be met via delivery from local blood vessels. Tumour cells must adapt to this reduced oxygen environment in order to survive. This is in part achieved through hypoxia-induced stabilisation of hypoxia-inducible factor (HIF) - a master transcription factor that activates a massive transcriptional cascade affecting multiple cellular and systemic processes. Many of these processes aid tumour growth, for example metabolic changes including a switch to glycolytic metabolism to support anaerobic ATP production; angiogenesis to support tumour growth and, potentially, metastasis. In addition, HIF may alter processes such as proliferation and apoptosis that are less obviously concerned with oxygen balance but which may impact tumour growth/survival.

Whilst it is well documented that activation of HIF target genes may facilitate tumour growth, it is less clear whether HIF can initiate cancer per se. The high incidence of genetic mutations in HIF pathway components in tumours provides some evidence for this. For example, von Hippel Lindau (VHL) - one of the major negative regulators of HIF - is a tumour suppressor and patients with germline mutations develop VHL syndrome, a familial cancer syndrome characterised by tumours in a restricted set of tissues: haemangioblastomas (spinal and cerebellar), retinal angioblastomas, renal clear cell carcinomas, pheochromocytomas and carotid paragangliomas. Given the role of VHL in both HIF regulation and as a tumour suppressor, this suggests that activation of HIF could drive tumourigenesis, at least in certain tissues.

Tumours of the adrenal medulla or carotid body, collectively termed pheochromocytomas/paragangliomas (PCC/PGL), not only have a high incidence of VHL mutations, but also have been shown to contain a number of gain of function mutations in HIF-2alpha (see recent review). Further, the carotid body is unique in that hypoxaemia, low arterial oxygen as experienced at altitude or in patients with chronic obstructive pulmonary disease (COPD), induces marked proliferation and overgrowth of the carotid body. This is thought to mediate ventilatory acclimatisation, an increase in ventilation in response to chronic hypoxia that helps redress oxygen balance. In line with this enhanced proliferation, the incidence of carotid body tumours, or carotid body paragangliomas, is ~10x more common at altitude/in COPD. Taken together, this suggests that HIF is capable of initiating tumourigenesis in sympathoadrenal tissues of the carotid body and adrenal medulla, perhaps via stimulation of proliferation.

Using transgenic mouse models, we have demonstrated that HIF-2 is necessary for hypoxia induced carotid body proliferation and the associated ventilatory acclimatisation. Further, we have shown that inactivation of the principle negative regulator of HIF: HIF prolyl hydroxylase enzyme 2 (PHD2) results in carotid body overgrowth with near 100% incidence of markedly enlarged, dysplastic carotid bodies with features characteristic of human PGL tumours and that this process is dependent on HIF-2 (unpublished data and 4).

The aim of this internship would be to characterise the mechanisms by which aberrant HIF-2 activation leads to the development of these PGLs. In the first instance, we would seek to understand which cellular processes (metabolic, secretory or other) are dysregulated; for example through a transcriptomic study of early HIF-2 dependent gene expression changes and through analysis of cellular features including dense core vesicle secretion. We would test whether these HIF-2 effects extend to other tissues, in particular those that develop tumours in VHL disease. From a clinical perspective, we would test whether pharmacological modulation using recently described HIF-2 antagonists can moderate the development of PGL, as has been described in renal clear cell carcinoma.

We anticipate that these mice will form a paradigm not only for the study of PGL tumours but also for other ‘pseudohypoxic’ cancers — that is, cancers associated with genetic mutations affecting hypoxia signalling such as renal clear cell carcinoma associated with inherited or sporadic VHL mutations.
References:

Professor Simon Davis

Influence of transmembrane domain sequences and their lipid modifications on protein-protein interactions in the membrane

Protein-protein interactions (PPIs) at the membrane govern the majority of signal transduction pathways within cells. These pathways can control cell growth, proliferation, apoptosis, motility and in the case of immune cells, their activation. A well-established example within immune cells is the triggering of the cell surface T cell receptor (TCR). Upon TCR binding ligand, the intracellular and membrane-associated kinase Lck associates with and phosphorylates the TCR (the ‘trigger’) leading to downstream signalling cascades, the release of internal calcium, and T cell activation. By attaching Lck to various TM domains with different amino acid sequence and lipid modification status, it is plausible interaction rates between TCR and Lck will now differ. Changes in interaction rates would translate into altered kinetics in signal transduction and therefore T cell activation. By measuring readouts of time to T cell triggering and activation, the influence of TM sequence and their lipid modifications can be addressed.

Techniques to be used
Cloning of membrane proteins, genetically engineering T cells, flow cytometry, confocal microscopy, T cell triggering and activation assays, and various tools for data analysis (e.g. FlowJo, Calquo, and Prism).

Investigator page: http://davislab-oxford.org/
Quicker vaccines for emergency response

**NOTE:** This is expected to be a longer-term project, a minimum of 3 months – your living costs will be supported for this additional length of time.

Traditional approaches to vaccine production involve designing a completely new bespoke vaccine and Good Manufacturing Practice compliant (GMP, i.e. clinical-grade) manufacturing process for each pathogen. This process takes years and is completely unsuitable to emergency responses to emerging pathogens. Following the recent Ebola and Zika epidemics, there is recognition of the need for ‘platform technologies’ which can readily be adapted to rapidly (within months) produce a new clinical-grade batch of vaccine for a previously unknown emerging pathogen. The adenovirus vector platform is well-suited to this in that it is capable of inducing an immune response to virtually any pathogen transgene, but the manufacturing process for each adenovirus vector is relatively similar.

Our group is working on developing a highly streamlined adenovirus purification method, based upon tangential flow filtration and ion exchange chromatography. This project will investigate some of the following as further improvements to the method:

- replacement of our current nuclease-based host-cell DNA removal method with a precipitation step, coupled with an HPLC-based quality control assay for removal of the precipitant.
- acceleration of the initial concentration and diafiltration stages using hollow fiber technology
- the possibility of removing the initial concentration and diafiltration stages, replacing them with a chromatographic capture step
- optimisation of the method to be suitable for multiple different adenovirus serotypes (which have different surface charge properties and hence behave differently in ion-exchange chromatography).

The project offers an unusual opportunity to learn about the requirements of designing a purification process suitable for making a clinical-grade product, while based within a strong academic environment. It would thus be particularly well suited to a student with an interest in working at the interface of pre-clinical and clinical research (i.e. laboratory work directly geared towards real-world impact), or with an interest in a future career in pharmaceutical biotechnology. It should however be of interest and benefit to students whose longer term interests lie outside these areas, in that it will provide a solid grounding in purification techniques which are applicable throughout protein biochemistry, structural biology and related areas.

**Techniques you will learn:**

- Mammalian cell culture (adherent and suspension)
- Tangential flow filtration
- Chromatography (using an Akta instrument)
- Quantitative PCR
- Basic virological techniques such as quantification of infectious virus by immunostaining.

**References:**


Investigator page: [https://www.ndm.ox.ac.uk/principal-investigators/researcher/alexander-sandy-douglas](https://www.ndm.ox.ac.uk/principal-investigators/researcher/alexander-sandy-douglas)
Professor Panagis Filippakopoulos

Protein:protein interactions leading to transcriptional repression

Transcriptional programs are often deregulated in disease, offering opportunities for therapeutic intervention. One of the most promising over recent years is through targeting epigenetic readers of the bromo and extra-terminal (BET) family. In this internship we seek to understand the contribution of the extra-terminal (ET) domain of BETs in the assembly of transcriptional complexes leading to repression of lineage-specific programmes using recombinant and cell biology techniques.

Investigator page: https://www.ndm.ox.ac.uk/principal-investigators/researcher/panagis-filippakopoulos

References:
Professor Pete Gething & MAP: 3 projects

Project 1: Quantifying the differences in rates of change in malaria incidence within countries

Malaria is a major source of death and disability but recent control efforts are drastically reducing the burden of malaria globally. However, it is not clear whether reductions in malaria incidence occur evenly across countries. This project aims to quantify the differences in rates of change of malaria incidence in high and low burden areas within a country. From a practical perspective, this is a vital step in our spatio-temporal models of malaria as current models cannot be fully flexible in both time and space. One solution is to fit an accurate spatial model and then adjust the estimates so that the national sums of malaria cases match estimates from more detailed time series models but exactly how this adjustment is done should mimic the differential changes across countries seen in the data. This project will entail analysis of our large database of routine surveillance data and fitted models will be incorporated into our ongoing work for the global burden of disease.

Project 2: Expanding the Malaria Atlas Project data R package

The Malaria Atlas Project has one of the largest databases of malarialometric data in the world. Recent efforts to make it easier for other groups to use our data have included an interactive, browser-based tool (https://map.ox.ac.uk/explorer) and an R package. The aim of this project is to extend the R package to enable dissemination of surveillance data. Surveillance data consists of counts of clinical malaria cases in a given time and administrative area. Therefore, the data needs to be associated with geographic polygons to be useful; this is a considerable technical hurdle. The new functionality in the package will be uploaded to CRAN to be used by malaria researchers across the globe.

Project 3: Mapping populations at risk of primaquine-induced haemolysis due to G6PD deficiency

*Plasmodium vivax* is the most geographically widespread human malaria species, due in part to its ability to form dormant parasites in the human liver that can relapse and cause repeated clinical malaria weeks to months after the mosquito inoculation and primary clinical episode. Primaquine is the only available drug to treat these liver-stage parasites and prevent the cumulative burden of relapses. However, for individuals with a common human enzyme deficiency (G6PD deficiency) using primaquine can cause serious adverse effects. This project will include a literature review & preliminary analysis of the epidemiological and clinical characteristics of G6PD polymorphisms across populations at risk of *P. vivax* infection in the Americas. The collected data will be used to produce maps of G6PD deficiency prevalence and variance, and meta-analyses will be conducted to refine our knowledge of the associations between G6PDd and *P. vivax* infection risk. Working with the Malaria Atlas Project, students will gain experience in conducting a systematic literature review according to PRISMA guidelines; creating and managing a database; using bibliographic management software; applying statistical analysis skills and mapping spatial data using GIS software.

Investigator page: https://www.ndm.ox.ac.uk/principal-investigators/researcher/peter-gething
Analysing the Prevalence of Infections Caused by Arboviruses in Mexico: Case Zika and Chikungunya

Zika virus is an emerging arthropod-borne virus of the family Flaviviridae that has spread rapidly around the world. In early 2015, Zika virus reached Brazil and a remarkable epidemic of Zika virus infections occurred in the Americas, spreading throughout the continent. The expansion to over 39 countries in the Americas in only two years has prompted the WHO to declare Zika virus an international public health emergency due to its link to congenital malformations and neurological complications. Equally important, Chikungunya virus is an alphavirus that co-circulate in most of the Zika endemic regions, where Dengue is prevalent.

At the Jenner Institute, the group led by Prof Arturo Reyes-Sandoval initiated a programme to develop a Zika vaccine alongside Dr Cesar Lopez-Camacho, postdoctoral scientist in his team. The development of this vaccine has allowed to plan for clinical trials (Phase I, Ib) in both the UK and Mexico.

Objective: To perform an epidemiological study of the laboratory confirmed cases of the outbreak in Mexico, using classical and molecular estimators for Zika and Chikungunya. This will allow to gather important data to support a Phase Ib clinical trial in Mexico.

Material and methods: Retrospective incidence of Zika and Chikungunya will be calculated using bona-fide databases. Data used will be comprising the following variables: sex, age, assigned status, date of onset of symptoms, predominant symptomatology, comorbidities and geographic location. A bioinformatics analysis will be also performed to support phylodynamics of the ZIKV and Chikungunya.

Investigator page: https://www.ndm.ox.ac.uk/principal-investigators/researcher/arturo-reyes-sandoval
Dr Chunxiao Song

Chemical biology approaches on epigenetic and epitranscriptomic modifications

Our genome is not a static state; it contains dynamic epigenetic modifications that play crucial roles from development to pathogenesis. Recently, many new nucleotide variants have been discovered in DNA and RNA, which triggered an explosion of new information in the epigenetics field. Chemical and biological transformations of nucleotide variants have always been the core of crucial methodologies in epigenetic research. Yet novel approaches are still needed to study many new modifications. In this project you will develop novel chemical and biological transformations on recently discovered epigenetic and epitranscriptomic modifications to fuel the research in epigenetics.

Investigator page:  [https://www.ndm.ox.ac.uk/principal-investigators/researcher/chunxiao-song](https://www.ndm.ox.ac.uk/principal-investigators/researcher/chunxiao-song)
Developing viral vectored vaccines for MAGE-expressing tumours

Cancer vaccines have the potential to induce anti-tumour specific immune responses to reject tumours. This project aims to produce and test new MAGE-targeting cancer vaccines optimized to induce strong cytotoxic T lymphocyte (CTL) responses. We are using an effective viral vector platform and different immunogen design to induce potent CTL responses against tumours. Different mouse tumour models are employed to assess the vaccine efficacy. This study will create a next-generation cancer vaccine against MAGE-positive tumours, and lay a foundation for clinical testing in cancer patients.

The project involves techniques in molecular biology and immunology.

Investigator page: [https://www.ndm.ox.ac.uk/principal-investigators/researcher/benoit-van-den-eynde](https://www.ndm.ox.ac.uk/principal-investigators/researcher/benoit-van-den-eynde)
Professor Frank von Delft: 2 projects

Project 1: Exploring ligand design for a ubiquitin-specific protease linked to Alzheimer's

The ubiquitin specific proteases (USPs) play a key role in the protein quality control system of the human proteome. As part of a collaboration with ARUK we are generating chemical probes using structural biology to discover molecules that maybe useful in the treatment of, or research into, Alzheimer’s disease. The aim of this short project is to generate a crystal structure of one of these candidate USPs with a bound ubiquitin molecule: the structure of this complex will be crucial to informing our small molecule design strategy. The project will involve expression/ purification and crystallization of the enzyme, generation of a reactive ubiquitin probe, and crystallization of it bound to a USP. Depending on time, you will also perform XChem crystallographic fragment screening with crystals of the complex.

Project 2: Automated synthesis in fragment-based lead discovery for a neurodegeneration target

Since 2015, the XChem facility (Diamond Light Source, UK) has shown the importance of fragment screening by X-ray crystallography, generating more than 1000 high quality fragment hits on over 50 disease related protein targets. However, only a handful have been progressed further to biological potency. Understanding ligand interaction is key for the development of an early fragment hit to a chemical probe. For this highly interdisciplinary project, you will work at the automated synthesis platform we are developing at Diamond, to synthesise 100s of follow-up compounds from several fragment hits discovered by XChem screen of NUDT7, an enzyme linked to neurodegenerative disease. Your work will include X-ray screening of the produced compounds, and possibly NMR, to assess the affinity of the compounds towards their target, and computational analysis of the crystallographic results.

Investigator page: https://www.ndm.ox.ac.uk/principal-investigators/researcher/frank-von-delft
Dr David Wedge

Timing the mutational processes that cause cancer

Cancers are caused by mutations, which result from various mutagenic processes, including errors during DNA replication, enzymatic modification of DNA and the effect of exogenous carcinogens such as ultraviolet light and tobacco\(^1\). Current areas of research are the identification of the underlying causes of mutations and of the ‘signatures’ that represent characteristic patterns of mutation resulting from each source. There is growing evidence that the mutational processes acting on cells change as cells transform from normal into cancerous cells and as tumours grow and spread. The proposed project will apply computational methods to identify these mutational process changes and the role they play in tumour growth and resistance to treatment.

Investigator page: [https://www.ndm.ox.ac.uk/principal-investigators/researcher/david-wedge](https://www.ndm.ox.ac.uk/principal-investigators/researcher/david-wedge)

References:

Associate Professor Wyatt Yue

The complexity of multi-protein complexes in metabolism

Living cells have evolved complex metabolic systems, where functionally related enzymes do not work in isolation but instead cluster into supramolecular complexes, to provide spatial and temporal control of key molecules in the cell.

A paradigm example is the Multi-Synthease Complex (MSC), composed of 8 aminoacyl-tRNA synthetases (ARSs) and 3 auxiliary proteins.

ARSs provide the set of enzymatic reactions required for maintaining the genetic code, and the coordination of ARSs within MSC are hence key to the integration of the entire translation process by the ribosome.

Our group is interested in studying the molecular architecture and assembly mechanism of MSC, which have thus far remained elusive despite its cellular importance.

This project will apply recombinant antibodies, immunoprecipitation, and chromatography techniques, in order to isolate the MSC from human cell lines for mass spectrometry, X-ray scattering and electron microscopy.

We have carried out small scale tests that demonstrated the feasibility of this approach to generate the multi-protein complex from endogenous cells.

Investigator page:  [https://www.ndm.ox.ac.uk/principal-investigators/researcher/wyatt-yue](https://www.ndm.ox.ac.uk/principal-investigators/researcher/wyatt-yue)