MRC Research Experience Placements Summer 2024

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Towards development of novel anti-cancer agents against oncogenic microRNAs: synthesis and assessment of their binding and catalytic activities

Supervisor: Elena Bichenkova
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Research profile: Elena Bichenkova

Significance and potential impact

A major biomedical challenge is highly-selective therapy against abnormal gene expression in disease states (e.g. cancer, inflammation) where combination therapies, including comparatively toxic drug cocktails, are otherwise indicated. Novel therapeutic strategies for selective treatment of disease states can be facilitated by targeting of upstream cellular components (e.g. messenger RNA, small non-protein-coding RNAs) to achieve controlled translational arrest of pathogenic proteins and thus trigger a desired therapeutic response. Indeed, short functional non-coding microRNAs are implicated in many types of cancer, and thus can be used as biological targets for development of more selective and powerful anticancer therapies to allow minimising adverse drug reaction and severe toxicity.

Aims of the project

This project will be focused on synthesis of the peptidyl-oligonucleotide hybrids to achieve selective targeting of oncogenic microRNAs (e.g. miR-21, miR-17, miR-155) with abnormal expression profiles in cancer. These chemically engineered RNA-targeting molecules will be generated by conjugation of short, catalytically inactive peptides with DNA recognition motifs to produce novel biologically-active molecules capable of recognising and cleaving disease-relevant RNAs. The most remarkable feature of these molecules is that conjugation of peptide and oligonucleotide building blocks synergistically combines the individual properties of the two components, and yields a new, hybrid molecule with unusual catalysis, capable of cleaving RNA molecules under physiological conditions.

Proposed research plan

The design of this type of novel biologically-active compounds will be based on the use of 3D structural data, which were previously gained in the host laboratory from computational modelling, and novel chemical strategies for site-directed conjugation. This will be followed by evaluation of the ability of the synthesised compounds to

hybridise and irreversibly destroy the fluorescently-labelled microRNAs in a sequence-specific manner. The main output of this project is to achieve a high-level of potency and reaction catalytic turnover while retaining effective bio-specificity.

Association between air pollution and cardiovascular diseases: an urban and rural comparative analysis

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Other information: SQUARE

This study will aim to assess and compare the prevalence of cardiovascular diseases in Urban and Rural residents exposed to indoor and outdoor air pollution. Findings from this study will help in employing strategies to increase the adoption of clean household energy, including advocacies and policies that provide support to purchase alternative cleaner technologies and fuels, improved ventilation or housing design, and communication campaigns to promote clean energy use.

Methodology

The candidate will be trained and supported to undertake a meta-analysis during the 6 weeks internship. This technique is used in research to systematically combine and analyse the results from multiple independent studies on a particular topic. Instead of focusing on individual study findings, a meta-analysis pools data from various studies to provide a more comprehensive and robust summary of the evidence.

Relevant publications

- Emekwuru, E. and Ejohwomu, O. (2023) Temperature, humidity and air pollution relationships during a period of rainy and dry seasons in Lagos, West Africa. Climate. https://doi.org/10.3390/cli11050113

- Ejohwomu, O.A., et al., (2022), The Exposure of Workers at a Busy Road Node to PM2.5: Occupational Risk Characterisation and Mitigation Measures. Int. J. Environ. Res. Public health 2022, 19(8), 4636; https://doi.org/10.3390/ijerph19084636

- Ejohwomu, O. et al, (2022) Modelling and Forecasting Temporal PM 2.5 Concentration Using Ensemble Machine Learning Methods. Buildings. https://doi.org/10.3390/buildings12010046

Novel measurement methods for non-regulatory emission species from fuel additives

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Fuel additives are proposed in many countries as a step towards improving the performance of engines in the transport and industrial sectors. There are thousands of fuel additives on the market, which have been developed by hundreds of companies worldwide. These additives have been used to improve the performance (such as the brake horsepower and fuel consumption) of internal combustion engines, and they are widely promoted. They are especially attractive for users of large fleets of commercial and heavy-duty vehicles, owners of high-performance vehicles, and during winter as additives to improve cold engine combustion.

However, surprisingly, only a limited number of studies exist on the emissions due to these additives and their consequent health effects. This is important because these additives will become increasingly popular as the global economy pushes towards massive reductions in and more efficient use of fossil fuels in the transport sector. It is crucially important that the environmental and health effects of these additives are ascertained at the early stages of development so that improvements in fuel consumption are not traded for increased hazardous emissions.

This area of study will not only shed light on this issue but will ultimately inform policy for future fuel additive developments. This project allows us to start to investigate the emissions from fuel additives and their health implications.

Methodology

The candidate will be trained and supported to undertake a meta-analysis during the 6 weeks internship. This technique is used in research to systematically combine and analyse the results from multiple independent studies on a particular topic. Instead of focusing on individual study findings, a meta-analysis pools data from various studies to provide a more comprehensive and robust summary of the evidence.

Longitudinal investigation of neuropathic progression in individuals with impaired glucose tolerance: a 10-year follow-up study

Supervisor: Maryam Ferdousi
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We propose a longitudinal study within this project, aimed at following individuals initially diagnosed with impaired glucose tolerance. Through extensive neuropathy assessment performed at baseline and upon diabetes onset, we aim to reveal the progression and impact of diabetes on neuropathic status. We plan to follow up with these patients over a 10-year period to assess the long-term implications of impaired glucose tolerance and diabetes on neuropathy.

The student's role will involve recording the participants' current neuropathy status by contacting their respective General Practitioners. Our goal is to gain insights into how impaired glucose tolerance progresses to diabetes and understand how this progression affects neuropathy. The student will have the valuable support and guidance of a group of experts, including Dr. Maryam Ferdousi, Dr. Shazli Azmi, and Professor Handrean Soran.

Do psychedelic drugs increase the connectivity between neurones in the brain?

Supervisor: John Gigg
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Aims

Psychedelics such as psilocybin are compounds with profound perception-, emotion- and cognition-altering properties. Psilocybin has been hailed as a "breakthrough therapy" for pervasive, difficult-to-treat neuropsychiatric conditions, particularly major depressive disorder. As such, psilocybin has unique potential to treat the causes of psychiatric illness and enable recovery (1, 2). The current hypothesis of how psychedelics improve cognition centres on their proposed ability to 'reprogram' the brain by reversing the downscaling of synaptic connections in affective states such as depression. However, the physiological mechanisms by which psilocybin 'reprograms' brain function and modifies synaptic connectivity are not understood.

Objectives. To reveal the neural and synaptic effects of psychedelics, the student will record ongoing activity from regions of the rodent brain during drug challenge with psilocybin. Recordings will target cognitively relevant regions including medial prefrontal cortex and hippocampus (3). Stimulating electrodes will also be used to activate synaptic input to these regions to monitor whether psilocybin increases baseline neurotransmission to modulate connection strength between neurones over the short- and/or long-term (seconds to hours). The student's project will fit within and form part of the project work of an MRC-funded PhD student in the lab.

Thus, the main questions for the summer project relate to the effect of psilocybin on the brain:

- Does psilocybin increase the power and coherence of signalling between brain regions? We will measure this as changes in the strength of theta and gamma rhythms; these EEG states are known to support synaptic plasticity and cognitive processing.
- Does psilocybin increase the strength of synaptic connections within brain regions over the short (seconds) to long term (minutes)? We will measure this as the ability of psilocybin to produce short- and/or long-term changes in effective connectivity between neurones (e.g., short-term synaptic facilitation and/or long-term synaptic potentiation).

Selected references

1. Nutt D et al. (2020) Psychedelic Psychiatry's Brave New World. Cell. 181:24-8.
2. Carhart-Harris RL & Friston K. (2019) REBUS and the anarchic brain: toward a unified model of the brain action of psychedelics. Pharm. rev. 71:316-44
3. Jin J and Maren S (2015) Front. Syst. Neurosci. doi: 10.3389/fnsys.2015.00170

Early detection of brain cancer using nanopore sequencing of DNA methylomes

Supervisor: Petra Hamerlik
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Approximately 16,000 people are diagnosed with a brain tumour in the UK every year and there are estimated to be over 60,000 people living with a brain tumour. Alarmingly, brain cancer is projected to be the 10th most common cause of cancer-related deaths for all age groups and genders in 2023.

Due to the relatively low incidence of brain tumours and the non-specific symptoms associated with intracranial disease, diagnosing brain tumours in primary care populations is rare. Recent studies have found that symptomatic patients with a brain tumour visit their GP an average of five times before being referred to secondary care, with up to 61% of brain tumour patients being diagnosed in an emergency setting, often following a seizure. Since the risk of recurrence and mortality are closely related to the disease stage at the time of primary intervention, late diagnosis is among the key factors contributing to the poor outcome of brain cancer patients.

Earlier diagnosis of brain tumours is crucial to minimise brain injury, subsequent disability, and late effects of treatment. Therefore, we are developing test which uses cell-free DNA methylomes for early detection of brain cancer based on liquid biopsies, tears in particular. The aim of this project is to optimise the isolation of cell-free DNA from Schirmer strip which is a strip of filtration paper used for collecting tears from patients. The student will work closely with a postdoctoral fellow and in collaboration with Oxford Nanopore technologies.

Beyond dopamine D2 receptor antagonism for the treatment of schizophrenia – myth or reality

Supervisor: Michael Harte
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Schizophrenia is a neuropsychiatric disorder with heterogenous symptoms which are divided into three main categories: positive symptoms (e.g. delusions, hallucinations, disordered thoughts), negative symptoms (e.g. social withdrawal, apathy, avolition), and cognitive dysfunction. A striatal hyperdopaminergia is proposed to underlie the positive symptoms with recent positron emission tomography (PET) studies revealing that dopamine levels are significantly elevated in the associative and sensorimotor regions of striatum but not in the limbic area in schizophrenia patients.

Currently approved pharmacological treatments (i.e. antipsychotics), through the blockade of dopamine D2 receptors, mainly target the positive symptoms with little efficacy for the cognitive and negative symptom domains. Even in treating the positive symptoms, the antipsychotic drugs yield less success than desired; the treatment is slow-acting and often results in a multitude of adverse effects. These side-effects vary depending on the antipsychotic drug prescribed but generally include weight gain, sedation, and extrapyramidal motor side effects.

Since the positive symptoms of schizophrenia are linked to an aberrant striatal dopaminergic signalling, it is not surprising that a common model for studying these positive symptoms is administering acute amphetamine to rodents. Recent findings in our lab indicate the presence of a pathophysiology in the sub-chronic phencyclidine (scPCP) model that potentially renders the model relevant to the positive symptomatology in schizophrenia.

The current study will utilise the scPCP model to further understand the pathophysiology that leads to aberrant dopamine signalling in the striatum with the overall aim to identify novel non-dopaminergic targets for the treatment of schizophrenia.

T cell invasion in 3D cancer spheres

Supervisor: Adam Hurlstone
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Research profile: Adam Hurlstone

Being able to engineer T cells to invade cancers will enhance their ability to suppress tumour growth. 3D in vitro models for measuring invasion will expedite the evaluation of strategies for increasing the invasiveness of T cells. The aim of the project, therefore, is to assist a graduate student to develop models for evaluating T cell invasion in 1) 3D matrices and 2) into spheroids comprising established cancer cell lines.

1. Transwell inserts will be coated with a layer of collagen and inserts filled with solution containing T cells. T cell invasion into a lower chamber containing chemokine medium will then be ascertained using counting beads and flow cytometry.

2. Cancer spheroids will be formed in ULA plates and then transferred into collagen gel containing T cells stained with vital dye. The spheroids will then be washed and the fluorescence they contain quantified on a plate reader and imaged with a fluorescent microscope.

An investigation of the brain-endothelial glycocalyx and TJP1, using an in vitro Blood-Brain Barrier model

Supervisor: Stavros Panagiotou
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The semipermeable blood-brain barrier (BBB) is responsible for monitoring and regulating the control of substances passing from the blood into the brain, including pathogens, neurotoxins, oxygen and water, as well as maintaining homeostasis within the central nervous system (CNS). Dysfunction of the BBB may occur due to disease, ageing or the disruption of essential compounds, such as tight junctions which forms between endothelial cells or the glycocalyx, a dense layer surrounding the vascular endothelium.

Tight junction protein-1 (TJP1) and syndecan-1 are components of the endothelial cellular cosmos. The glycocalyx is responsible for maintaining the stability of its surrounding environment and TJP1 and syndecan-1 assist in maintaining its integrity. TJP1 limits the intercellular space between endothelial cells and regulates the size and charge of molecules attempting to cross the BBB. Syndecan-1 plays a vital role in reducing inflammation and studies suggest that changes in its expression can disrupt the BBB.

Aims and objectives

The present study aims to investigate the effects of TJP1 and syndecan-1 expression when the BBB is challenged by acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein, varicella zoster virus (VZV) or Streptococcus pneumoniae.

An in vitro co-culture BBB system will be established using endothelial and astrocyte cells, to assess endothelial barrier integrity during different infection scenarios. Immunohistochemistry will allow us to measure the expression of TJP1 and syndecan-1 under the aforementioned conditions.

Assessment of blink dynamics using a mobile phone

Supervisor: Michael Read
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Research profile: Michael Read
Other information: Eurolens Research

In recent years blinking has received increased attention across multiple disciplines. Blinking has been suggested as an indicator of cognitive load, psychological state, fatigue, neurological diseases and ocular conditions. Traditional methods to record blinking have primarily involved electromyography and magnetic search coils, which are considered invasive and complex techniques. Recently, the use of high-speed cameras has overcome some of the technical difficulties of traditional methods allowing a less invasive assessment of blinking.

Given the potential for blinking to act as a biomarker of ocular and systemic disease, there exists a need for more efficient and automated methods to analyse human blinking. We developed a novel non-invasive high-speed infra-red imaging system, as well as custom semi-automated software that extracts blink metrics from video recordings. This system has been employed in several studies which have evaluated a range of clinical conditions, including contact lens wear, diabetes and digital eye strain.

This research has identified blink metrics as an important objective biomarker of ocular comfort and has highlighted the potential for blinking to be used as a biomarker of systemic disease processes, such as peripheral neuropathy. However, the cost of high-speed cameras is not insignificant. With advances in technology, many currently available mobile phones can record high-speed videos, which can potentially be used to capture the blinking process. The use of mobile phones could make the assessment of blinking more accessible to clinicians and researchers.

This project aims to investigate the assessment of blinking using a mobile phone (i.e. 240 frames per second) in comparison with our high-speed infrared imaging system (i.e. 500 frames per second). Participants will be recruited, and their blinking will be assessed using these two methods simultaneously. Videos will be analysed using semi-automated software to extract blink metrics and determine the agreement between methodologies.

Genetic variants and diagnostic criteria assessment in heterozygous familial hypercholesterolemia: a study from Manchester Royal Infirmary's Lipid Clinic

Supervisor: Handrean Soran
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Heterozygous Familial Hypercholesterolemia (HeFH) is the most common monogenic cause of raised serum cholesterol, affecting about 1 in 250 to 1 in 500 people (1-3). It is dominantly inherited. Affected family members have LDL cholesterol levels typically double those of unaffected first degree relatives. Serum cholesterol level more than 4.9 mmol/L in adults with family history of premature ASCVD raises the possibility of HeFH and LDL-C is commonly 9–12 mmol/l (348–464 mg/dl). It is higher from birth and HeFH can be diagnosed in childhood. Untreated it results in tendon xanthomata typically in the Achilles tendons (Achilles tendon pain may be first manifestation) [3] and extensor tendons on the dorsum of the hands. Subperiosteal xanthoma are also sometimes present on the tibial tuberosities. FH is caused by pathogenic variants in LDL receptor, apolipoprotein, apolipoprotein E and PCSK9 genes.

CVD occurs with increasing frequency from the third decade so that without medical intervention over half of affected men and 15% of affected women die before the age of 60 years [3]. National Institute for Health and Clinical Excellence (NICE) recommends screening for FH and diagnose using genetic testing followed by family cascade testing (4).

To assess the frequency of pathogenic variants in each gene, pre-treatment LDL cholesterol level in patients who had genetic testing for HeFH is Manchester Royal Infirmary's lipid clinic. Validity of using Simon Broome criteria compared, Dutch Lipid Clinics Network Criteria and an LDL-C cut off will also be assessed.

Circulating PCSK9 concentration in patients with type 1 diabetes

Supervisor: Handrean Soran
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Other information: EndoDiabLipid.com

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is expressed in many tissues and contribute to cholesterol metabolism and regulation. The PCSK9 protein is secreted chiefly by the hepatocyte, with smaller quantities being expressed by the intestinal epithelia, nervous tissue [1] and renal mesenchyme [2]. PCSK9 secreted by the liver primarily controls circulating serum LDL cholesterol levels. PCSK9 prevents the LDL receptor from translocating to the cell surface by either acting as a ligand to cell-surface LDL receptors post secretion, or by binding directly to nascent LDL receptors pre-secretion mediating lysosomal destruction of the LDL receptor [3]. In addition to background statin treatment,PCSK9 monoclonal antibodies reduce low-density lipoprotein cholesterol (LDL-C) and cardiovascular signoficantly with an excellent efficacy and safety profile [4].

PCSK9 level is higher in patients with systemic inflammation, statin treated and hypercholestrolaemia. However, it is not known if PCSK9 concentration is higher in patients with type 1 diabetes and if circulating concentration correlates with LDL cholesterol level.

Hypothesis

Circulating PCSK9 concentration is higher in patients with type 1 diabetes (non-statin treated) compared to healthy controls.

Method

To compare data available in our research laboratory on free and bound PCSK9 concentration in patients with type 1 diabetes and healthy controls.

Project delivery

The student is expected to:

- perform a literature search on PCSK9, its physiology and PCSK9 in type 1 diabetes;
- establish a database for patients with type 1 diabetes and controls including demographics, PCSK9 and lipid profile results;

- analyse the data to explore the above hypothesis.

Early detection and differential diagnosis of dementias via functional neuroimaging and machine learning

Supervisor: Jason Taylor
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Research profile: Jason Taylor
Other information: Manchester Neuroimaging - EEG lab

Alzheimer's disease (AD) causes deterioration of brain regions that normally support memory function. One aspect of memory that is affected early in AD is semantic memory, one's knowledge of concepts and word meanings. We have developed a cognitive task that is sensitive to subtle impairments of semantic memory when combined with measurements of brain activity using electroencephalography (EEG) and functional MRI. These changes in brain function may be apparent even before changes to brain structure can be seen on MRI scans, which means they could be used to diagnose AD earlier. Our goal is to develop software tools that can automatically determine whether brain activity during this semantic task shows signs of AD using machine learning (ML).

We are currently collecting data for a patient-facing study collecting EEG and fMRI data from mild AD patients and healthy controls (HC) whilst they perform several simple semantic tasks. The Research Experience Placement student will be involved in the continued operation of this study. The student will primarily be required to assist in the identification and recruitment of HC participants (N<=15) through online platforms, the administration of basic neuropsychological assessments, and the collection of EEG data during several cognitive tasks. Training will be provided by the research team at the EEG lab. Secondary to recruitment and data collection, the student will have the opportunity to support the research team in the management, processing and analysis of existing fMRI and EEG data.

Project timeline

- **08/07-15/07**: Training in EEG data collection, neuropsychological assessment administration and the use of online recruitment platforms.
- **15/07-12/08**: Participant recruitment and data collection. 3-5 participants per week @ 2hrs per participant. Data management, basic data analysis.
- **12/08-15/08**: Production of a short presentation and summary of the EEG and behavioural data, to be presented to the research team.

Identifying cis-regulatory elements governing neural crest development, with implications in neural crest derived birth defects

Supervisor: Ruth Williams
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Research profile: Ruth Williams

The neural crest is an embryonic stem cell-like population that contributes to a remarkable range of tissues in the vertebrate body, including craniofacial cartilage and bone, neurons and glia of the peripheral nervous system and the cardiac outflow tract and septal. Consequently, errors in neural crest patterning, migration, and differentiation result in a wide range of congenital birth anomalies collectively termed neurocristopathies, these account for almost one third of all birth defects.

This project aims to identify novel cis-regulatory interactions underlying the activity of genes known to be involved neural crest development and implicated in neurocristopathy aetiology. This will involve exploring existing epigenomic data sets, in particular; ATAC-seq generated from neural crest cells isolated from developing chick embryos and single-cell Multiome data generated from early human cranial tissue, for putative cis-regulatory elements (enhancers) driving key neural crest genes. Putative enhancers will then be cloned into a fluorescent reporter plasmid and tested for enhancer activity in vivo using the chick embryo. Fluorescent in situ hybridisation will also be used to detect expression of candidate genes in developing chick embryos. The student will therefore learn some bioinformatics skills as well as molecular biology, embryology, and imaging techniques.

Identifying enhancers driving neural crest development will further resolve the gene regulatory network governing this crucial developmental process. Furthermore, we will determine the conservation of chick neural crest enhancers in the human data, which will allow us to infer putative enhancer activity in human and thus provide novel loci at which to search GWAS and patient data sets for causative SNPs underlying neurocristopathy aetiology and determine enhancer pleiotropy in during neural crest development and disease.