EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Ribosomal glycopeptide production to investigate glycosylation changes in prostate cancer

School of Natural and Environmental Sciences, Newcastle University

Supervisory Team

- **Dr Tom McAllister, Newcastle University (Lead)**
- **Dr Jennifer Munkley, Newcastle University**
- **Dr Clare Mahon, University of Durham**

Project overview/context

Changes in glycosylation of proteins are hallmarks of disease, but the underlying molecular mechanisms of these processes are not fully understood.
This project will use synthetic chemistry to produce a range of glycosylated amino acids that will be combined with peptide mRNA-display to generate a glycopeptide display method to completely cover protein sequence space. This will be used to identify the substrates of disease-related enzymes such as the glycosyltransferase GALNT7, which is upregulated and drives tumour growth in prostate cancer.

Research Project

Background:
Glycosylation in the Golgi of eukaryotic cells produces a vast array of heterogenous glycans (sugar-modified biomolecules) with at least 10 different modifications of proteins identified to date.[1] These protein modifications have a myriad of functions, including regulating protein interactions, altering protein structure or providing recognition motifs. Serine and threonine residues can be modified by multiple sugars (N-acetylgalactosamine (GalNAc), N-acetylglucosamine, glucose, fucose, mannose and xylose), creating very complex structures. Glycans with an initial O-GalNAc have the most diversity with 100s of different structures identified to date, with expression levels of different biosynthetic enzymes impacting the final glycans produced and interplay between different glycosylation sites.

Glycans change dramatically in prostate cancer and are linked to a malignant phenotype.[2] The Munkley group have recently discovered that the *N*-acetylgalactosminyl transferase GALNT7 promotes tumour growth in prostate cancer.[3] GALNT7 acts upon glycopeptides[4] but comprehensive information about its substrates is not known. Determining its substrates will further our understanding of disease progression and help improve diagnosis and treatment.

Workplan:

1. Synthesis of glycosylated amino acids

Using established methods to perform glycosylation reactions on amino acids to produce conjugates at the mono-, di- and tri-saccharide levels.

2. Glycopeptide synthesis

The glycosylated amino acids will be charged on to tRNAs for *in vitro* translation reactions; incorporation into ribosomal peptides will be optimised.

3. Screening glycopeptides by mRNA-display

Libraries of glycopeptides covering all possible sequences[5,6] will be produced and screened for substrate activity using GALNT7. Identification of substrates will be achieved through sequencing the mRNA-appended to each peptide and data combined to provide insight into the substrate selectivity.

4. Substrate validation/characterisation

Chemical synthesis using in-house approaches to produce a selection (100s) of putative substrate peptides and validation using *in vitro* enzymatic assays. This can be extended to identify potential GALNT7

substrate proteins within the human proteome using the peptide motifs discovered and these validated using cell culture models.

Summary:
Prostate cancer is the most common cancer in men, impacting 1 in 8 men within their lifetime.[7] There is an urgent unmet clinical need to improve how prostate cancer is diagnosed, and also to develop new treatments for advanced disease. Thus, new approaches to study the molecular basis of glycan production both generally and in prostate cancer as highlighted here are needed.

References:
1) Cold Spring Harb Perspect Biol. (2011) Apr 1;3(4):a005199;
2) Int J Mol Sci. 2019;20(6).
3) Oncogene (2023), 42, 926-937
4) FEBS Lett (1999). 460:226–230
5) Pep. Sci. (2021) 113 (1), e24204
6) Chem. Sci (2018), 9 (20), 4569-4578
7) CA Cancer J Clin. 2016;66(1):7–30

Training & Skills

The student will be part of a team of experienced chemists, chemical biologists and molecular/cell biologists based in the McAllister group, situated in the recently refurbished state-of-the-art chemical biology laboratories in the Bedson building at Newcastle University. The project will provide essential training to the student in:

- Organic synthesis with a focus on carbohydrate and amino acids/peptide chemistry
- Molecular biology; biochemical and biophysical assays, cell culture
- Excellent, broad appreciation of modern chemical biology approaches
- Transferrable skills in scientific methods, management and leadership through the MoSMed CDT training programme

Overall the student will obtain a wide skillset, which could form the foundations for a further career in chemical biology or medical science.

Further Information

For enquiries, please contact Dr Tom McAllister tom.mcallister@newcastle.ac.uk

How to Apply

If applying to a **Newcastle project**, you must apply through the University's **Apply to Newcastle Portal**. Once registered select **'Create a Postgraduate Application'.**

Please note, at this time we can only accept applications from students who qualify for UK/home fees for this project. Please see the URKI website for more details.

Use 'Course Search' to identify your programme of study:

- search for the 'Course Title' using the programme code: **8207F**
- select '**PhD Molecular Sciences for Medicine (SNES)'** as the programme of study

You will then need to provide the following information in the 'Further Questions' section:

- a 'Personal Statement' (this is a mandatory field) - upload a document or write a statement directly into the application form. Please include the full title of the studentship, the studentship code, and how your interests and experience relate to the project.
- the relevant studentship code **(mos23_08)** in the 'Studentship/Partnership Reference' field. If you wish to apply for additional studentships, please make sure to add the relevant studentship reference each time, before submitting each separate application. For example, you may wish to apply for mos23_08 AND mos23_13. **You must include the relevant code for your application to be considered.**
- when prompted for how you are providing your research proposal - select 'Write Proposal'. You should then type in the title of the relevant research project. You do not need to upload a research proposal.
- An up to date CV.
- Please upload all documents in PDF format.

Equality, Diversity and Inclusion (EDI)
Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the MoSMed application process to Newcastle University please contact Craig Hinds, the MoSMed CDT Manager: **mosmed.cdt@newcastle.ac.uk**

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