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

p53 function and metal levels in cancer cells

Durham University, department of Biosciences in collaboration with Biosciences, Newcastle University and Pleco Therapeutics

Supervisory Team	• **Lead: Patricia Muller, Dept of Biosciences, Durham University** • **Co-supervisor: John Lunec, Newcastle University** • **Industrial Partner: Pleco Therapeutics**

Project overview/context

P53 is the most important protein preventing tumour formation. In many cancers, this protein is damaged and altered. Importantly, an altered p53 protein does not protect against cancer, but promotes cancer formation and spreading of the cancer. Despite this altered protein being an obvious therapeutical target, no drugs are available for routine use against altered p53. In cancers, metal levels are often increased. We have discovered that such metal levels change p53 function. In this project we will generate a metal and p53 sensor.

Research Project

Many cancers have increased metal levels. Metals are used as cofactor for enzymes needed for enhanced cell growth during tumour formation.

In this project we will explore the correlation between p53 folding status and intracellular metal levels by developing a p53 function and metal sensor. We will validate the use of the sensor and then explore if we can find therapies that can impact on metal levels and p53 function simultaneously.

Pivotal for this project will be to correlate p53 function to the amount of available metals in the cells. For this purpose, we will develop sensor assays to detect metal levels and p53 function simultaneously. Metal sensing will be based on the sensor developed in the Lead supervisor's PhD lab (PMID: 17617060). This sensor is responsive to metals through the metal response elements cloned from the promoter of the metal transcription factor MTF1. As an example, we will generate a vector that combines an MRE luciferase reporter with a p53 responsive renilla reporter separated by a linker to express in equimolar amounts. We will generate stable cell lines expressing this construct and use it to detect which metals affect p53 function (increased luciferase/ renilla ratio). These assays will be performed in the presence of a p53 activator, nutlin. Metal accumulation in cells exposed to this mix will be measured (ICP-MS) and p53 function (RT-PCR, apoptosis) and folding (immunoprecipitation or FACS with folding Antibodies) validated.

After validation of the sensor, we will assess which therapies work best to lower the luciferase/ renilla ratio in cells exposed to metals. P53 is activated by DNA damage induced by chemotherapy. We will study this using not only the reporter assay, but also cell survival assays.

The lab of supervisor 2 at Newcastle University is interested in both DNA damage induced and non-genotoxic activation of p53 using MDM2 and WIP1 inhibitors that promote p53 stabilization by preventing degradation. Instead of using chemotherapy, the student will spend time in this lab to use the MDM2 inhibitors.

This project therefore provides the exciting possibility to develop a new assay to study metal levels in combination with p53 function and to apply this to a setting in which this is most relevant.

Training & Skills

The student will be trained in a variety of techniques needed for this project. These include but are not limited to tissue culture, transfections, cloning, luciferase assays, metal measurements, immune precipitation, western blot, qRT PCR and FACS analysis. In addition, the student will be part of the Durham PhD program and will be allowed to follow courses, the seminar program and progress will be monitored on a 6 monthly basis.

How to Apply

To apply for this project please visit the Durham University application portal to be found at: Home · Application Portal (microsoftcrmportals.com)

Please select the course 'PhD in Molecular Sciences for Medicine (EPSRC CDT)', which is registered in the Chemistry Department and indicate the reference **MoSMed22_14** in the 'Field of Study' section of the application form. Please note that there is no need to submit a Research Proposal with your application, however we do require a Covering Letter, CV, academic transcripts, the contact details of two referees and proof of English language proficiency if relevant.

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 application process at Durham University please contact the Durham MoSMed CDT Manager, Emma Worden at: emma.worden@durham.ac.uk

Further Information

Please contact the Lead supervisor Dr Patricia Muller for further information:
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