TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title Does ENPP1 regulate bone mineralisation via altered mitochondrial function?

Lead PhD supervisor (please provide name, affiliation and email)
Vicky MacRae, The Roslin Institute, University of Edinburgh vicky.macrae@roslin.ed.ac.uk

Second PhD supervisor (please provide name, affiliation and email)
Mathew Horrocks, School of Chemistry, University of Edinburgh mathew.horrocks@ed.ac.uk

Background

Land mammals need to manage a constant supply of energy in an environment where food availability and intake can vary dramatically. In order to do this, they have evolved intricate networks of circulating hormones that coordinate fuel production and utilisation in specific organs of the body. Insulin, a hormone whose main function is to regulate blood glucose levels, is a major controller of energy utilisation. The maintenance of the skeleton is characterised by alternating phases of destruction and formation by specialised bone cells and has a high energetic cost, with the entire human skeleton being replaced every 10 years. Recent studies have revealed that the skeleton is able to coordinated whole body energy utilisation through its hormonal interactions with other tissues (Clemens and Karsenty 2011). Unravelling the pathways of this process will immediately improve our understanding of the control of energy balance and guide new approaches for the treatment of bone diseases and diabetes.

Our laboratory has generated exciting data revealing for the first time that mice lacking the enzyme Nucleotide pyrophosphatase/phosphodiesterase 1 (*Enpp1*$^{-/-}$) show extensive bone mineralisation defects; exhibit an insulin sensitive metabolic phenotype and are protected from high fat diet-induced obesity compared to control ("normal") mice (Huesa et al., 2014). This data has lead us to believe that NPP1 is specifically acting in bone forming cells called osteoblasts, to regulate energy utilisation through altering the function of mitochondria. Mitochondria are crucial organelles for energy generation. During a cell's lifetime, the mitochondrial network is continuously shaped by fission and fusion events, which underpin mitochondrial elongation and shortening. The dynamin-related GtPases optic atrophy 1 (OPA1) of the inner mitochondrial membrane, and mitofusins (MFN) 1 and 2 of the outer membrane, regulate mitochondrial fusion. Mitochondrial fission is controlled by cytosolic dynamin-related protein 1 (DRP1).

This PhD project, will undertake studies in the laboratory to investigate the role of mitochondria in bone mineralisation in detail. Specifically this project will determine if ENPP1 ablation in primary osteoblasts results in altered cellular metabolic function through analysis of mitochondrial respiration, morphology and mitophagy.

Aims

Aim 1:
This project will apply machine learning protocols (Fischer et al. iScience 2020 23: 101601) to a super-resolution microscopy approach to comprehensively assess mitochondrial morphology with nanometre resolution in *Enpp1*$^{-/-}$ and Wildtype (WT) osteoblasts. In parallel, established laboratory techniques will be used to induce and assess mineralisation in *Enpp1*$^{-/-}$ and WT osteoblasts (Huesa et al., 2014), including qPCR, western blotting and immunofluorescence staining.

Aim 2:
Assessment of fixed cells by super-resolution microscopy with DNA-PAINT (Jungmann et al. Nat Methods 2014 11:313–814) will determine spatial expression and location (with nanometre precision) of individual mitochondrial fission/fusion regulators in in *Enpp1*$^{-/-}$ and WT osteoblasts. Mitochondrial function will be investigated through biochemical assays.

Aim 3:
Functional studies will establish the individual effects of knock out or overexpression of OPA1, MFN1/2 and DRP1 on in *Enpp1*$^{-/-}$ and WT osteoblast mineralisation, through CRISPR-Cas9 gene editing/activation protocols.

Training and experience provided

The project will generate large datasets spanning the scales of the skeletal system from single proteins to the tissue-wide scale. We will use cross-cutting laboratory and data analysis techniques to ask whether ENPP1 plays a role skeletal mineralisation and insulin sensitivity via altered mitochondrial function. The student will be trained to tackle fundamental scientific questions in a vibrant atmosphere in three laboratories with complementary and wide-ranging expertise using state-of-the-art methods in biophysics.

The successful PhD student will receive training throughout their studentship, much of it through practical application of research techniques, analytical methods and study skills. Skills that will be acquired in the academic supervisors' labs include cell and molecular biology techniques, microscopy, cell culture and in vivo techniques. Students are also encouraged to attend and contribute to the monthly Research Workshops dedicated to student and post-doctoral presentations and the yearly student poster session. The University of Edinburgh's transferable skills programme will also be available to the student, with courses available in commercialisation,

research management, communication skills, time management, networking and team working available.

Expected outcomes

The student will be encouraged to present their work at International and European scientific conferences. Past students of the academic supervisors' of this project have typically published at least 3 scientific papers during their studentships, and have subsequently secured high profile post-doctoral research positions in the UK, Europe, USA and Australia.

References

Clemens TL, Karsenty G. 2011 The osteoblast: an insulin target cell controlling glucose homeostasis. J Bone Miner Res. 26: 677-80.

Fischer CA, Besora-Casals L, Rolland SG, Haeussler S, Singh K, Duchen M, Conradt B, Marr C. 2020 MitoSegNet: Easy-to-use Deep Learning Segmentation for Analyzing Mitochondrial Morphology. iScience. 23(10):101601.

Huesa C, Zhu D, Glover JD, Ferron M, Karsenty G, Milne EM, Millan JL, Ahmed SF, Farquharson C, Morton NM, MacRae VE 2014 Deficiency of the bone mineralization inhibitor NPP1 protects against obesity and diabetes. Dis Mod Mech 7: 1341-50.

Jungmann R, Avendaño MS, Woehrstein JB, Dai M, Shih WM, Yin P. 2014 Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. Nat Methods. 11(3):313-8.

Mackenzie NC, Huesa C, Rutsch F, MacRae VE 2012 New insights into NPP1 function: Lessons from clinical and animal studies. Bone 51: 961-68.