TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Investigating the role of Enpp1 deficiency in osteoporosis.

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Background

Osteoporosis is a condition that weakens bones and increases the risk of fractures. Patients with ENPP1 deficiency are prone both to fracture risk and to osteoporosis. In this project, we will use *in vivo* and *in vitro* mouse models to assess the role of Enpp1 deficiency in osteoporosis, and the potential benefits of metformin treatment. This builds on recent clinical reports revealing that metformin treatment reduces the incidence of osteoporosis (Cai et al., 2024).

The ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) enzymatic family consists of seven human enzymes, all of which generate small molecules that function as extracellular factors that regulate essential physiologic processes (Roberts *et al.*, 2019). Members of this enzymatic family regulate whole organism fate in mammals through essential biological pathways that include angiogenesis, cell motility, tumour metastasis, bone mineralization, vascular calcification, and haemostasis. ENPP1 was the first of these enzymes identified and is the only human enzyme capable of generating extracellular pyrophosphate (Maulding *et al.*, 2022).

Previous studies in our group have assessed the detailed changes in bone development in mice lacking Enpp1 (Enpp1-/-). We demonstrated that Enpp1(-/-) mice are characterized by severe disruption to the architecture and mineralization of long-bones, as well as dysregulation of calcium and phosphate homeostasis (Mackenzie *et al.*, 2012).

In this project we will employ the cutting edge technique of longitudinal [18F]NaF PET/CT to assess changes to the skeleton of this transgenic mouse line *in vivo* over a defined time-course. This imaging work will be complemented by downstream molecular analyses of plasma and tissues, as well as mechanistic cell culture studies. Finally, we will assess using metformin as a potential therapeutic strategy to protect against osteoporosis in our animal model, and undertake cull culture studies to investigate potential mechanisms.

Aims

Aim 1: Assess the natural history of the progression of osteopenia in the Enpp1 mouse model.

WT and Enpp1-/- male and female mice will be studied over an 8-week period from 4 weeks of age to 12 weeks of age. We will record weight and specific clinical phenotypes weekly. Additionally, we will replicate the clinical assessment of human patients by employing longitudinal [18F]NaF PET/CT

assessment of the skeleton at 8, 10 and 12 weeks of age. At 12 weeks of age, femoral bone architecture will investigated *ex vivo* using micro-computed tomography. The molecular phenotype of the plasma (e.g. plasma pyrophosphate (PPi), FGF23, osteocalcin and C-terminal telopeptides of type I collagen levels) will also be assessed at 12 weeks of age. Western blotting and qPCR analysis will be undertaken on tibiae to determine changes in expression patterns of key mineralisation regulators.

Aim 2: Assess efficacy of therapeutic intervention with metformin in the Enpp1 null mouse.

Here we will assess the effect of metformin treatment (subcutaneous administration; 50mg/kg in methylcellulose/tween-20) on (i) specific clinical phenotypes; (ii) *[^{18}F]NaF* PET/CT assessment of micro-calcification deposition within the aorta and heart; (iii) femoral bone architecture and (iv) the molecular phenotype of the plasma and (v) molecular expression patterns of mineralisation regulators.

Aim 3: Understanding the molecular mechanism of metformin action on osteoblasts.

Based on our recent studies in vascular cells (Phadwal *et al.*, 2022), the key molecular pathway that we expect metformin to act on to provide benefit is the autophagy pathway. This will be further explored using *in vitro* culture of an osteoblast-like cell line.

Training and experience provided

The student will be trained to tackle fundamental scientific questions in a vibrant atmosphere in laboratories with complementary and wide-ranging expertise using state-of-the-art imaging methods (longitudinal [18F]NaF PET/CT assessment, micro-CT analysis), a range of molecular techniques (e.g. qPCR, western blotting, cell culture) and in vivo mouse studies. They will also be encouraged to present work at UK and International scientific conferences and to undertake relevant professional and transferable skills training.

Expected outcomes

Past students of the supervisors' have typically published at least 2 scientific papers during their studentships, and have secured high profile post-doctoral or industry positions.

References

1. Cai Y, Jun G, Zhuang X. Metformin treatment reduces the incidence of osteoporosis: a two-sample Mendelian randomized study. Osteoporos Int. 2024 Jun;35(6):1089-1098.

2. Maulding ND, Kavanagh D, Zimmerman K, Coppola G, Carpenter TO, Jue NK, Braddock DT. Genetic pathways disrupted by ENPP1 deficiency provide insight into mechanisms of osteoporosis, osteomalacia, and paradoxical mineralization. Bone. 2021 Jan;142:115656.
3. Mackenzie NC, Zhu D, Milne EM, van 't Hof R, Martin A, Darryl Quarles L, Millán JL, Farquharson C, MacRae VE. Altered bone development and an increase in FGF-23 expression in Enpp1(-/-) mice. PLoS One. 2012;7(2):e32177.
4. Phadwal K, Koo E, Jones RA, Forsythe RO, Tang K, Tang Q, Corcoran BM, Caporali A, MacRae VE. Metformin protects against vascular calcification through the selective degradation of Runx2 by the p62 autophagy receptor. J Cell Physiol. 2022 237(11):4303-4316.
5. Roberts F, Zhu D, Farquharson C, Macrae VE. ENPP1 in the Regulation of Mineralization and Beyond. Trends Biochem Sci. 2019 Jul;44(7):616-628.