TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Understanding the pathogenesis of osteoarthritis by studies on osteocytes in subchondral bone using zebrafish as a disease model.

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Background

Osteoarthritis (OA) is a complex degenerative disease and a leading cause of disability in older adults. It affects over 500 million people worldwide, with estimates that about 70% of the population over 70 suffer from OA. Although with the reality of an increasing ageing population, the health and economic burden caused by OA is likely to compound. Osteoarthritis is an area of significant unmet clinical need and there are no disease modifying treatments for OA, unlike many other musculoskeletal diseases. It is therefore important to unravel the molecular mechanisms of OA to develop new treatments for this debilitating condition. But, where to look for clues to start with?

While the degradation of articular cartilage defines OA, it is the subchondral bone beneath that serves as the primary nutrient source for the cartilage which is avascular, and which is not innervated by neurons. **Changes in subchondral bone remodelling often precede cartilage degradation, correlating with increased innervation and pain, the hallmark symptom of OA.** Understanding the mechanism driving subchondral bone remodelling in OA may provide insights for designing future therapies to tackle the early stages of OA and prevent disease progression.

Our laboratory is focused on studying **osteocytes, the most abundant cells of our skeleton and master regulators of bone remodelling.** Osteocytes are very interesting cells. They live completely buried in the hard bone matrix, but developed efficient ways to regulate bone remodelling via a dense neuron-like network formed by cellular dendritic process. The formation of these cell processes dictates osteocyte function to regulate bone remodelling and bone homeostasis. We argue that underpinning molecular mechanisms of osteocyte function, specifically of their dendrite formation, will lead to therapeutic targets for osteoarthritis.

We use **zebrafish** as animal models. Zebrafish are freshwater bony fish used to model human bone diseases. They offer rapid development, transparency, regenerative capacity, and the availability of an arsenal of genetic tools to study bone cells. Aged zebrafish have OA like in humans. Moreover, zebrafish have bones with osteocytes and bones without osteocytes, which allows for studying bone remodelling dependent and independent of osteocytes in the same individual. To translate

findings from a fish model to function in humans, we also use human cell culture. Our findings will provide solid knowledge into osteocyte function in OA.

Aims

We will perform functional studies pre-selected genes expressed in osteocytes and neurons that are identified in large-scale genetic populational studies (Genome-Wide Populational Studies, GWAS) as associated with OA. These genes will be tested using zebrafish knockouts and cell culture. Our goal is to answer whether neuronal genes play a role in the formation of dendrites in osteocytes and in risk susceptibility to OA.

Aim 1. Bone remodelling and Osteoarthritis assessment in zebrafish mutants. Pre-selected genes have been mutated in zebrafish using CRISPR/Cas9. Here, we will characterise skeletal changes (bone and cartilage) in these mutants using routine laboratory techniques, such as bone and cartilage staining, TRAP staining, micro-computed tomography (uCT), and histological analysis. Different time points of zebrafish development will be used to assess changes before and after osteocytes formation. Osteocyte numbers, shape and vitality will be analysed using high resolution synchrotron micro-computed images and confocal microscopy. This aim will answer how some of the selected impact osteocyte function and OA.

Aim 2. Molecular mechanisms of dendrite formation: Concomitantly, pre-selected genes will be genetically modified in human mesenchymal cell line using CRISPR/CAS9. Cellular morphology and behaviour changes will be followed during mesenchymal to osteocyte differentiation. Transcriptomic analysis will be performed to identify molecular pathways involved in dendrite formation. This aim will provide translational support from findings in fish to humans, and will reveal molecular pathways involved in osteocyte dendrite formation.

Aim 3. Pharmacological targeting to disrupt and reverse phenotype. We will use drugs to target the gene of interest and pathways involved in dendrite formation using cell culture and zebrafish knockout. We will analyse whether the drug reverse the osteoarthritis knockout phenotype in zebrafish and the morphological changes found in cell culture.

Training and experience provided

This project will provide multidisciplinary training that includes:

- A preclinical in vivo model organism (Zebrafish), including genetic engineering and genetic modification, molecular biology, and phenotypic analysis of skeletal changes using diverse methodologies (Kague Lab)
- Imaging techniques: stereo and compound microscopes, confocal microscopy, micro-computed tomography and high-resolution synchrotron micro-tomography (Kague Lab)
- Imaging analysis (Kague Lab)
- Cell culture and genetic manipulation (Kague and Ralston Labs)
- Transcriptomic analysis (Ralston Lab)
- Understanding of bone diseases, including clinical aspects, molecular bases and translational implication of findings from basic research to the clinic (Ralston Lab)

Expected outcomes

We expect to uncover molecular mechanisms driving the formation of dendritic process of osteocytes, and their role in OA progression. We will validate genes identified from Genome-Wide Association Studies in the susceptibility to OA. We will generate animal and cellular models for

studying OA, osteocytes and to test potential therapeutics. We will uncover potential druggable molecular pathways to target osteocyte function to reverse OA progression.

References

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