Project Details	
Project Code	MRC23IIARBr Carroll
Title	Investigating senescence in osteoarthritis
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	The accumulation of senescent, 'zombie' cells cause tissue dysfunction and human disease including osteoarthritis (OA). This PhD project will investigate how growth, metabolism, and survival pathways are different between senescent and healthy cells. We will then explore how senescence contributes to OA pathology and whether we can prevent or reverse it.
Description	Cellular senescence has important physiological roles in tissue repair and during embryogenesis. Increased accumulation of senescent, or 'zombie' cells however causes tissue dysfunction and directly contributes to disease, including osteoarthritis (OA). Genetic and pharmacological clearance of senescent cells has been shown to alleviate OA pathology in a number of mouse models. These proof-of-principle studies suggest that compounds that can kill senescent cells (senolytics) represent novel therapeutic options to treat OA. To have any hope of developing senolytics, we need a better understanding the biology of senescence in vitro and in vivo. This project will develop our recent work characterising the mTORC1-autophagy pathway, how it contributes to senescence and importantly represents a targetable vulnerability for senescent cell survival. At present however, it is not clear whether the phenotypes we observe in multiple in vitro models of senescence are shared in vivo. The aim of this PhD project will be to fully characterise the mTORC1-autophagy pathway in senescence, specifically in in vitro and in vivo models of OA. The student will determine what a senescent cell 'looks like' at the molecular and cellular level in vivo, how the senescent cell number and specific, defined phenotypes change with OA development. mTORC1 and seenscence will be assessed in models with knockout of genes associated with OA risk. Lastly, the student will explore whether the clearance of senescent cells reduces OA severity. Objectives: 1. Identify the molecular mechanisms of mTORC1-autophagy dysregulation in senescence Using multiple musculoskeletal cell culture models of senescence, the student will interrogate the mechanisms regulating mTORC1-autophagy, including the ability to sense mitogenic signals such as growth factors involved in musculoskeletal function and regeneration e.g. TGFβ. Genes implicated in OA risk, identified by collaboration with Jon Tobias and Ben Faber (Bristol Medical School) will be knocked out using CRISPR/Cas9 (5-10 candidate genes) and followed up by characterisation of senescence. A variety of standard molecular cell biology techniques including cell culture, qPCR, Western blot and immunofluorescence microscopy will be utilised. 2. Characterise senescence in vivo Immunohistochemical analysis of a large catalogue of fish models (already available) will be used to characterise the number, cell type and location of senescent cells in the musculoskeletal system of animals with evidence of OA. Via collaborations at Sheffield and Edinburgh, we will import reporter fish lines for the senescence markers, p21 and p16INK4a (cyclin-dependent kinase inhibitors). They will be crossed with accelerated OA models, autophagy and mTORC1 reporter lines. For proof-of-principle

	experiments and optimisation of tools, fish will also be irradiated to induce premature senescence. Senescent cells will be isolated from tissue using laser capture microdissection. RNA-sequencing and proteomics analysis will be carried out alongside samples of senescent cells from culture to directly compare senescence in vitro and in vivo. Alongside, the student will also use a more targeted approach to explore changes in expression and splicing of regulators of mTORC1-autophagy pathway in senescence. 3. Can targeting the mTORC1-autophagy pathway have senolytic potential in vivo? We have shown in vitro that targeting the mTORC1-autophagy pathway has senolytic activity. To test this in vivo, a screen will be carried out in p21/p16 fish ~3dpf post-irradiation to induce widespread senescence. Published senolytics will be tested alongside mTORC1-autophagy modulators as positive controls.
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