| Project Details | |
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| Project Code | MRC22IIARBr Stephens |
| Title | Primary cilia assembly, disassembly, and cell proliferation in the context of wound repair. |
| Research Theme | Infection, Immunity, Antimicrobial Resistance & Repair |
| Summary | Primary (non-motile) cilia are hair-like extensions present on almost all animal cells that act as antenna for extracellular signals. Recent data has identified a key role for ciliary signalling in healing wounds and bone fractures. Primary cilia could be attractive targets to intervene in fibrosis and scarring. We have ambitious plans to use in vitro biochemistry, high resolution microscopy, and phosphoprotoemics to explore this opportunity. |
| Description | Primary (non-motile) cilia are hair-like extensions present on almost all animal cells that act as antenna for extracellular signals and are fundamental to proper metazoan development and ongoing health. In animals, primary cilia are required for key signalling pathways including hedgehog and TGF beta. Cilia extend from the mother centriole which precludes it use information of a mitotic spindle. Therefore, extension of cilia and entry into mitosis are, in most cases, mutually exclusive. Defects in cilia are linked to many inherited human diseases and more recent data has identified a key role for ciliary signalling in wound healing including resolving bone fractures. This has led to the proposal that assembly and disassembly of primary cilia and ciliary signalling could be attractive targets to intervene in fibrosis and scarring. Our work has focussed on the role of the dynein-2 motor in assembly and function of primary cilia. We were first to define the composition of a metazoan dynein-2 motor complex, human cytoplasmic dynein-2, and have used genome engineering to demonstrate the function of key subunits within this motor in cilia assembly and function. Our work has now defined the centriolar subdistal appendage protein CEP170 as the key site for assembly of the ciliary transport machinery. Importantly, CEP170 is constantly expressed throughout the cell cycle but phosphorylated during mitosis by kinases including Polo-like kinase 1 (Plk1) and Tank-binding kinase (TBK1). These are known to control CEP170 function in both interphase and mitosis but their impact on cilia function is unknown. Here we propose a project based on the expertise of the Stephens and Wakefield labs in primary cilia biology and mitosis respectively, with training and implementation of cutting-edge bioimaging methods, including super-resolution light microscopy and 3D electron microscopy, combined with in vitro reconstitution experiments and phosphoproteomics. These technologies provide fantastic training opportunities for the student as well as ample opportunity to shape and define the scope of the project as they wish. We will support the student to obtain training in-house and through external courses such as those provided by EMBO. Our hypothesis is that CEP170 defines the location for the assembly of the ciliary machinery. We also have evidence that it plays an important role in ciliary disassembly – a prerequisite for cell cycle entry. Thus, we propose that the CEP170-dynein-2 axis provides a critical point of integration of centriolar function and the decision between ciliary function and cell cycle entry. Genome editing, selective kinase inhibition, and recombinant protein expression provide |

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| | opportunities to perturb the system. We can then link these outcomes to key features of cell biology in the context of wound healing including proliferation, migration, and signal transduction. While amenable to work in diverse 2D and 3D cell culture models, we will also develop the use of chondrocyte and osteoblast lineages to provide relevant context to this work. In vitro work will be led by purification of centrosomes from mammalian cells for which established protocols exist. A further route to develop this project lies in the direction of population health sciences data. In our in vitro work we have defined a series of proteins not known to be associated with ciliopathies. It is therefore feasible to consider interrogating GWAS data to develop this work. Chrissy Hammond Is a co-lead of the EBI Mechanisms to Populations strand and will co-supervise the student providing support here and in the context of bone fracture healing. In terms of career preparation, we have had previous contact with pharma, including AstraZeneca, who are interested in the potential of dynein-2 as a drug target. We will continue to explore impact development through this project; this of course also links to a possible "Broadening Horizons" placement. |
| Supervisory Team | |
| Lead Supervisor | |
| Name | Professor David Stephens |
| Affiliation | Bristol |
| College/Faculty | Life Sciences |
| Department/School | Biochemistry |
| Email Address | david.stephens@bristol.ac.uk |
| Co-Supervisor 1 | |
| Name | Professor James Wakefield |
| Affiliation | Exeter |
| College/Faculty | College of Life and Environmental Sciences |
| Department/School | Biosciences |
| Co-Supervisor 2 | |
| Name | Dr Chrissy Hammond |
| Affiliation | Bristol |
| College/Faculty | Life Sciences |
| Department/School | PPN |
| Co-Supervisor 3 | |
| Name | Dr Hermes Gadelha |
| Affiliation | Bristol |
| College/Faculty | Engineering |
| Department/School | Engineering Mathematics |
| Co-Supervisor 4 | |
| Name | |
| Affiliation | |
| College/Faculty | |
| Department/School | |