CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Analysing the role of MYO1F in glioblastoma**
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Project Outline	**Aims and objectives** The actin cytoskeleton is essential for cell adhesion, migration and polarity, and changes in its dynamics and architecture are linked to cancer progression, malignancy and metastasis. Myosins are molecular motors that translocate along actin filament tracks and produce movement and force powered by ATP. The human genome contains 39 myosin genes that can be grouped into 12 classes based on their sequence similarity. In most normal but also in cancerous mammalian cells multiple myosins are expressed and several classes have been shown to play key roles in regulating tumor progression and metastasis (1). In this project we will focus on MYO1F, a Class I myosin, which functions at the membrane-cytokeleton interface, providing mechanical force and tension that allows remodelling of membranes relative to the underlying actin network (2). The MYO1F tail directly associates with cell membranes through a positively charged lipid-binding region and a pleckstrin homology (PH) domain and in addition contains an SH3-domain and a proline-rich region for protein-protein interactions. MYO1F is selectively expressed in peripheral myeloid and lymphoid cells and in brain-resident glia cells. Loss of MYO1F causes deficiencies in peripheral myloid cells with defects in extravasation, the migration of leukocytes into peripheral tissues (3) and defects in the deformation of neutrophil nuclei through physical barriers in 3D environments (4). Interestingly, recent studies using RNAseq-based gene co-expression have identified MYO1F as a glioblastoma-specific gene, which is up-regulated in glioblastoma relative to normal brain and lower grade glioma samples (5). MYO1F is one of the 22 genes separately identified in two gene expression data sets and high expression of these genes is associated with decreased survival linked to mesenchymal glioblastoma. Although very little is known about the precise cellular functions of MYO1F, our preliminary data indicates that this myosin is localised to and regulates podosomes/invadopodia formation in peripheral macrophages and microglia. In cancer cells, invadosomes promote cell migration and matrix degradation, therefore correct localization and assembly of these structures is likely to be crucial for tumour cell invasion. Myosin motors are attractive drug targets and small molecule inhibitors have already been identified for the myosins in classes I, II, V and VI. We currently receive funding through a BBSRC project grant to develop small molecule inhibitors for previously untargeted myosins, including MYO1F. We have established a project with Dr Susan Boyd (CompChem Solutions Limited, Cambridge), an expert in computational drug design, to widen our repertoire of compounds based on our previously identified myosin inhibitors such as PCIP. Our preliminary analysis shows that the binding site for PCIP on MYO1C has a low degree of sequence conservation with MYO1F (33% sequence identity), which should be sufficient to enable structure-based design approaches towards more selective compounds.

Experimental plan	A. The aim of the MRes rotation project is to verify the link between MYO1F overexpression and high grade glioblastoma (1-3): 1. Test MYO1F expression in molecular subtypes of glioblastoma and glioblastoma stem cells (6). 2. Determine the localisation and distribution of MYO1F in glioblastoma cell lines using MYO1F antibodies and ectopic expression of tagged MYO1F 3. Reduce MYO1F expression in glioblastoma cell line using siRNA KD or CRISPR CAS9 gene editing and investigate the effect on cell migration, adhesion and invasion using a variety of established assays. B. Future approaches and techniques for a PhD project include: 4. Identify the MYO1F interactome in glioblastoma cell lines using quantitative proteomics and in situ proximity labelling. 5. Characterisation of MYO1F adaptor protein using a variety of approaches 6. Screen potential new inhibitors for MYO1F for their efficiency in inhibiting MYO1F dependent processes in peripheral immune cells 7. Test and characterise MYO1F inhibitors for their affinities and effects on actomyosin kinetics *in vitro* using in vitro motility assays and stopped-flow kinetics.
Main Techniques	• Immunoblotting • Immunofluorescence and analysis using different microscopy techniques • siRNA KD and CRISPR CAS9 editing • BioID and quantitative mass spectrometry • Characterisation of novel MYO1F interactors using a variety of biochemical methods • Design and screening of small molecule MYO1F inhibitors
Key References	1. Ouderkirk JL, Krendel M.Non-muscle myosins in tumor progression, cancer cell invasion, and metastasis. Cytoskeleton (Hoboken). (2014) 71(8):447-63 2. McIntosh BB and Ostap EM.Myosin-I molecular motors at a glance. J Cell Sci. (2016) 129(14):2689-95. 3. Kim SV, Mehal WZ, Dong X, Heinrich V, Pypaert M, Mellman I, Dembo M, Mooseker MS, Wu D, Flavell RA. Modulation of cell adhesion and motility in the immune system by Myo1f. Science. (2006) 314(5796):136-9. 4. Salvermoser M, Pick R, Weckbach LT, Zehrer A, Löhr P, Drechsler M, Sperandio M, Soehnlein O, Walzog B.Myosin 1f is specifically required for neutrophil migration in 3D environments during acute inflammation.Blood. (2018)131(17):1887-1898 5. Dunwoodie, et al. Discovery and validation of a glioblastoma co-expressed gene module. Oncotarget (2018), Vol 9 10995-11008 6. Xie et al., The Human Glioblastoma Cell Culture Resource: Validated Cell Models Representing All Molecular Subtypes. EBioMedicine, 2 (2015) 1351–1363