Imaging the cytoskeleton in health and disease

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Introduction

Our main research interests focus on the cytoskeleton, and in particular two of the three types of filamentous systems: microtubules and actin filaments. Microtubules are important for trafficking of proteins, RNA, and vesicles throughout the cell, and in forming the scaffold for chromosomal organisation and movement during mitosis. They are associated with two families of molecular motors: kinesins and dyneins. Actin Filaments are also important for shorter range trafficking, cell shape, motility and contractility, and they are associated with the myosin family of molecular motors. Our research this year has ranged from investigating the roles of myosins in prostate cancer metastasis, to understanding how a deacetylase enzyme (HDAC3) modulates the acetylation status of microtubules, together with developing super-resolution light microscopy approaches to better visualise protein organisation and dynamic behaviour in cells.

Results

Specific myosins are important in prostate cancer metastasis: We investigated myosin expression and localisation in prostate cancer cells with low and high metastatic potential, and discovered that several myosin isoforms (Myo1b, Myo9a, Myo10 and Myo18) were expressed at higher levels in more highly metastatic cells. SiRNA mediated knockdown had distinct effects on the organisation of the acto-myosin cytoskeleton, cell shape and migration for each specific myosin (Fig. 1). Particularly striking was the large increase in actomyosin bundles that appeared when the expression of Myo18a was knocked down (Fig. 1). Our results nicely demonstrate how each myosin plays a distinct role in the cell, and can contribute to the metastatic phenotype (Makowska et al., 2015).

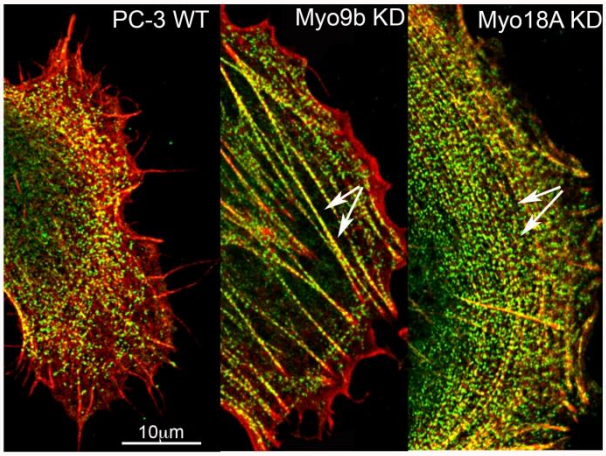

Figure 1: Organisation of actin (red) and non-muscle myosin 2A (green) in prostate cancer (PC-3) cells before (WT) and after knockdown of Myo8b and Myo18a. The arrows show the distinct re-organisation of acto-myosin bundles in the knockdown cells.

Super-resolution Imaging.

We have now built a new super-resolution microscope, the iSIM (instant structured illumination microscope Curd et al., 2015) in collaboration with Dr Hari Shroff at the NIH. This complements our existing PALM/STORM (photoactivated light microscopy/stochastic optical reconstruction microscopy) instrument. While PALM and STORM can improve resolution by ~10-20 fold compared to a standard light microscope, the iSIM allows a 2-fold improvement in resolution, but very fast image capture (up to 100 frames per second). We have used the STORM set-up to image protein organisation in primary cilia, capturing the fine detailed organisation within these small structures (Lambacher et al., 2016).

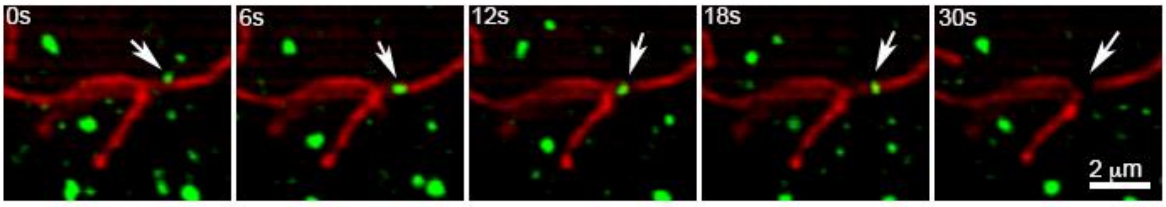

Figure 2: iSIM images of mitochondria (red) and Dynamin related protein 1 (DRP1 in green), shows the association of DRP1 with the mitochondrion, followed by possible fission of the mitochondrion (image taken with Fiona Hartley, UG project student).

Other projects:
We are continuing to work on the structure and function of single alpha helical domains (BBSRC funded), beta-cardiac myosin mutations in heart and skeletal muscle disease (MRC and BHF funded).

Publications
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