



Group Leader
Michael Olson

Research Scientists
Felipe Lourenco¹
Michael Samuel

Scientific Officer
June Munro

Graduate Students
Grant Wickman
Jenifer Wood

¹ funded by National Cancer Institute (USA)

Ras and Rho signalling pathways are frequently deregulated in human cancer; consequently tumour cells are stimulated to proliferate, resist apoptotic stimuli and metastasise. Our lab is currently studying how Ras and Rho regulate these cellular responses. Specifically, we are examining the role of Rho effector proteins in proliferation, invasion and metastasis using *in vivo* and *in vitro* methodologies. Through these studies we aim to identify critical proteins in these signalling pathways that are potential cancer therapeutic targets.

Many roles of the actin cytoskeleton

A major function of the actin cytoskeleton is to provide the structural underpinning that gives a cell shape and mechanical strength. The actin cytoskeleton is dynamic, undergoing constant rearrangement and reorganisation in response to external factors. Alterations to the cytoskeletal architecture have significant consequences on the entire cell - such as morphology, cytokinesis, adhesion and motility - but also at the subcellular level. As well as these structural functions, the actin cytoskeleton additionally acts as a scaffold, bringing together proteins that not only contribute to cell shape but also proteins that have diverse activities such as signal transduction and gene transcription. The scaffolding function is not limited to the spatial organisation of protein complexes as the actin cytoskeleton may also recruit or stabilise specialised membrane domains.

As described above, the actin cytoskeleton plays critical roles in gross cellular activities. However, it has also been shown that the cytoskeleton influences numerous fundamental processes including proliferation and cell death. A major challenge is to determine the mechanisms by which the actin cytoskeleton may have effects on these seemingly more distal biological functions.

The Rho family of small molecular weight GTPases consists of 20 members. Regulation of the actin cytoskeleton is a common activity of this group of proteins, although the fine details of how they affect actin structures and the proteins

that mediate these responses differ between individuals. Now an intensive area of research, Rho GTPases have been shown to be major players in many diverse biological processes. In numerous cases, the pathways leading from individual Rho proteins to the machinery of actin regulation have been relatively well-characterised but the means by which Rho proteins affect cell proliferation or cell death are not as clearly defined.

ROCK-induced cellular tension and tissue stiffness

The Rho/ROCK signalling pathway is frequently activated in tumours by direct actions of regulatory proteins, by upstream signalling oncogenes, or by growth factor receptors activated by mutation or increased ligand levels. Biophysical studies have also revealed that extracellular forces evoke mechanoreciprocal cellular tension *via* Rho/ROCK activation to balance internal and external forces. In parallel, cellular contractile force may further increase extracellular matrix stiffness via reorganisation of matrix structures and stimulation of matrix protein deposition, thereby creating a self-reinforcing mechanical autocrine loop. Recently, it has become appreciated that the frequent increased tissue stiffness associated with tumours is not a passive bystander but actively promotes cancer. Therefore, a significant challenge is to determine how the development of cellular tension may be both a cause and effect of tissue stiffness and how this is translated into tumour promotion. To determine how actomyosin

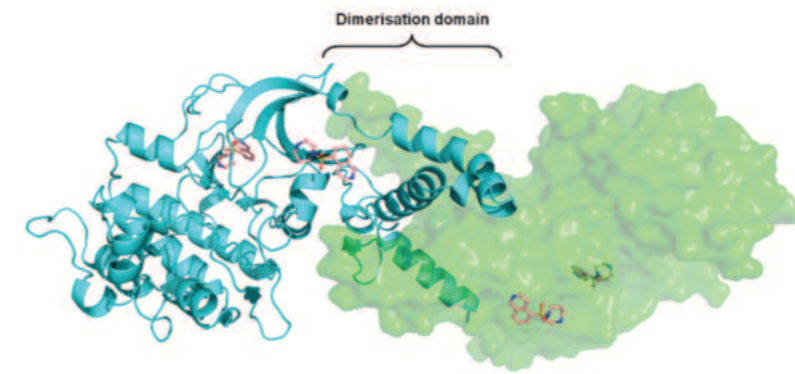


Figure 1
Overall structure of the dimeric MRCKβ shows the interactions of the two monomers at the dimerization domain. The four Fasudil molecules observed per asymmetric unit are also shown.

tension affects tissue homeostasis and tumour development, we expressed conditionally-active ROCK2 in mouse skin. ROCK activation elevated tissue stiffness via increased collagen density and deposition. β-catenin, a key element of mechanotranscription pathways, was stabilised by ROCK activation leading to nuclear accumulation, transcriptional activation and consequent hyperproliferation and skin thickening. Blocking the downstream F-actin regulator LIM kinase inhibited these responses. Skin tumour number, growth and progression were increased by ROCK activation, while ROCK blockade was inhibitory, implicating actomyosin-mediated cellular tension as a significant tumour promoter. These findings illustrate how a key GTPase signalling intermediate frequently activated in human cancer can promote tumour growth and progression by modulating the interplay between cell and tissue level forces.

LIM kinases in tumour cell invasion

LIM kinases 1 and 2 (LIMK1/2) are centrally positioned regulators of actin cytoskeleton dynamics. Using siRNA-mediated knockdown or a novel small molecule inhibitor, we found LIMK is required for path generation by leading tumour cells and non-tumour stromal cells during collective tumour cell invasion. LIMK inhibition lowers cofilin phosphorylation, F-actin levels, serum response factor transcriptional

activity and collagen contraction, and reduces invasion in three-dimensional invasion assays. Although motility was unaffected, LIMK inhibition impairs matrix protein degradation and invadopodia formation associated with significantly faster recovery times in FRAP assays indicative of reduced F-actin stability. When LIMK is knocked down in MDA-MB-231 cells, they lose the ability to lead strands of collectively invading cells. Similarly, when LIMK activity is blocked in cancer-associated fibroblasts, they are unable to lead the collective invasion of squamous carcinoma cells in an organotypic skin model. These results show that LIMK is required for matrix remodelling activities for path generation by leading cells in collective invasion.

RhoC and LIMK2 in cell survival

The central arbiter of cell fate in response to DNA damage is p53, which regulates the expression of genes involved in cell cycle arrest, survival and apoptosis. Although many responses initiated by DNA damage have been characterised, the role of actin cytoskeleton regulators is largely unknown. We found that RhoC and LIMK 2 are direct p53 target genes induced by genotoxic agents. Although RhoC and LIMK2 have well-established roles in actin cytoskeleton regulation, our results indicate that activation of LIMK2 also has a pro-survival function following DNA damage. LIMK inhibition by siRNA-mediated knockdown or selective pharmacological blockade sensitised cells to radio- or chemotherapy, such that treatments that were sub-lethal when administered singly resulted in cell death when combined with LIMK inhibition. Our findings suggest that combining LIMK inhibitors with genotoxic therapies could be more efficacious than single-agent administration, and highlight a novel connection between actin cytoskeleton regulators and DNA damage-induced cell survival mechanisms.

Publications listed on page 81

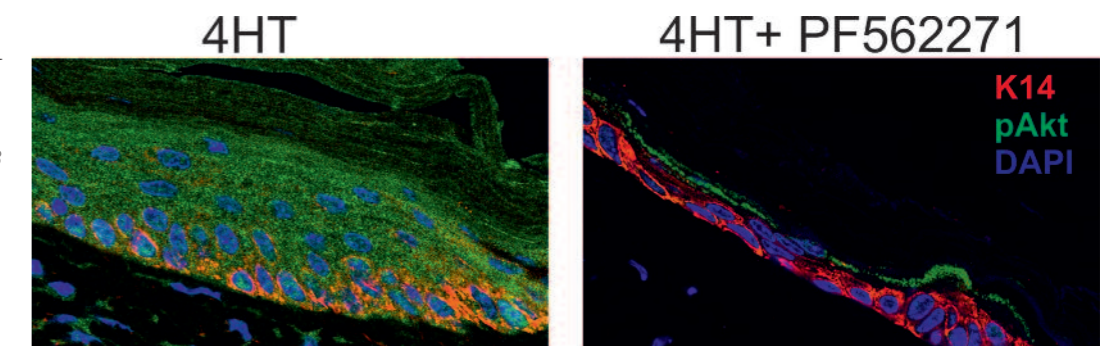


Figure 2
Effect of conditional ROCK2 activation with 4HT in skin stained for Keratin 14 (red) and Akt pSer473 (green) without or with FAK inhibitor PF-562271 treatment.