

Title: Elucidating the role of ROCK in the biomechanics of pancreatic tumour cells

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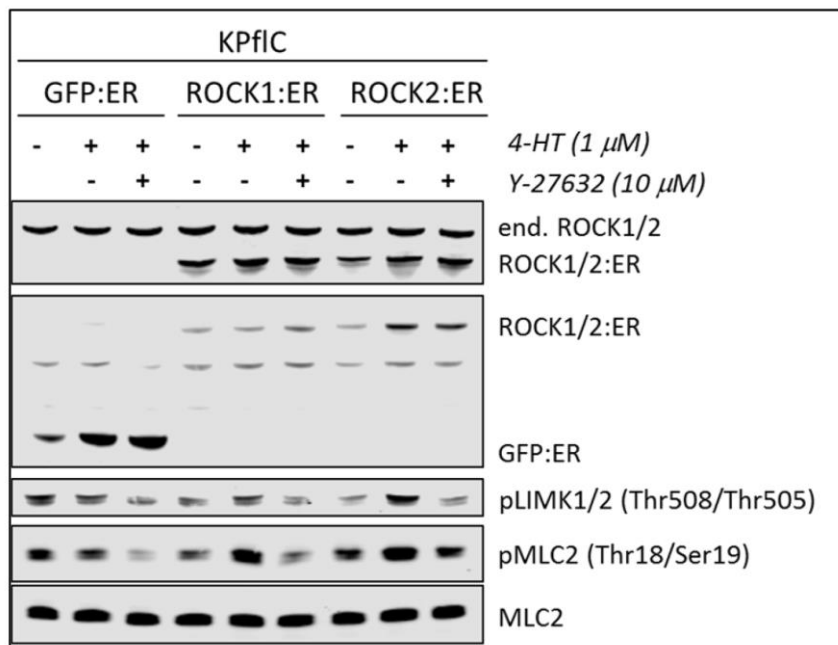
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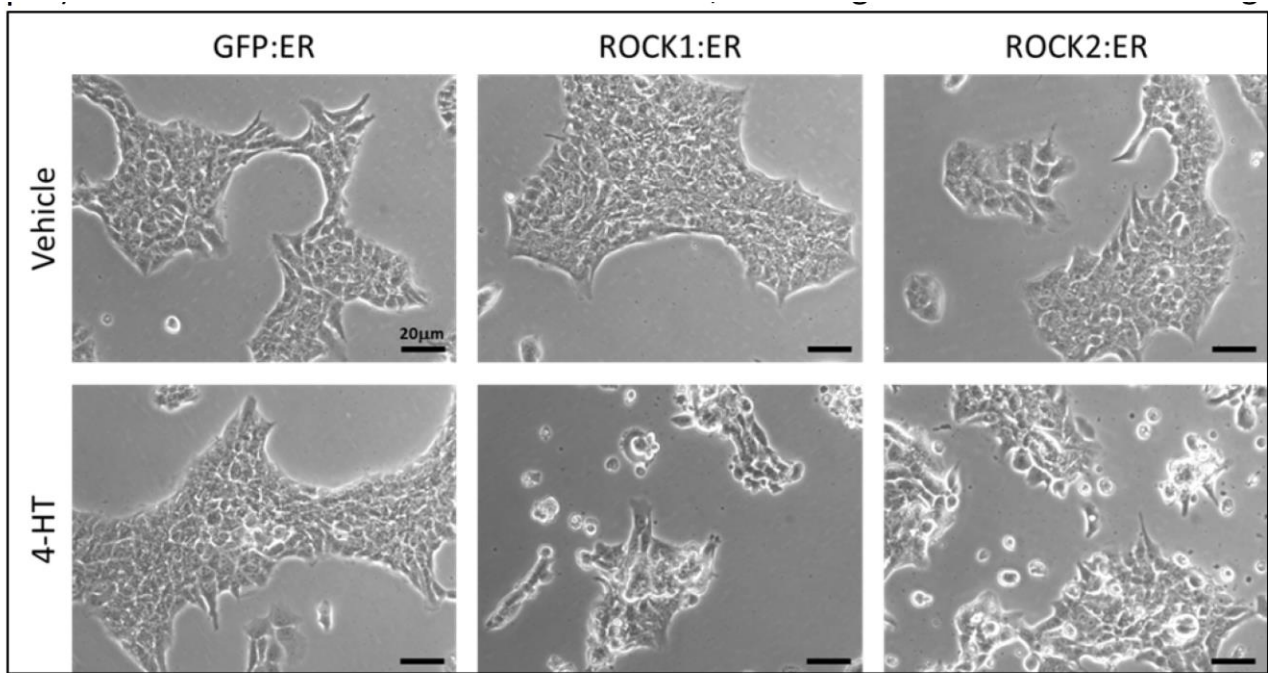
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The ROCK1 gene was found to be amplified in ~15% of human pancreatic tumours [1]. To determine how increased ROCK signalling might contribute to pancreatic cancer, we (Olson laboratory) expressed conditionally-activatable ROCK1 or ROCK2 fusion proteins that are coupled to the hormone-binding domain of the estrogen receptor (ER) [2] in mouse pancreatic tumour cells that express KRas G12D and are homozygously deleted of p53 (KpflC). Using this system, we can selectively activate ROCK activity by treatment of cells with estrogen analogues such as 4-hydroxytamoxifen (4-HT). In contrast to KpflC cells expressing control GFP:ER, treatment of ROCK1:ER or ROCK2:ER cells for ~18 hours with 1 μ M 4-HT increased the phosphorylation of the ROCK substrates LIMK1/LIMK2 and MLC2, which could be inhibited by coadministration of the ROCK selective inhibitor Y-27632 (10 μ M).



Consistent with a role for ROCK in regulating cell morphology, treatment of ROCK1:ER and ROCK2:ER cells with 4-HT (1 μ M) for ~18 hours resulted in cell contraction, rounding and membrane blebbing.



The student will choose to work on one of these two questions:

- (1) How much contractile force is generated by ROCK activation?
- (2) Does ROCK activation alter mechanosensing?

Techniques to be used: magnetic tweezers, traction force microscopy, elastic pillars sensors

References

1. Biankin, A.V., Waddell, N., Kassahn, K.S., Gingras, M.C., Muthuswamy, L.B., Johns, A.L., Miller, D.K., Wilson, P.J., Patch, A.M., Wu, J., et al. (2012). Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature* 491, 399-405.
2. Croft, D.R., and Olson, M.F. (2006). Conditional Regulation of a ROCK-Estrogen Receptor Fusion Protein. *Methods Enzymol* 406, 541-553.