Kidney Disease Associated Mutation (T119I) Alters Myosin 1e Intracellular Localization

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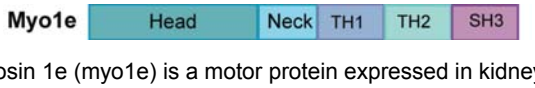
Background



Myosin 1e (myo1e) is a motor protein expressed in kidney cells. It contains a head, neck, and 3 tail homology domains.

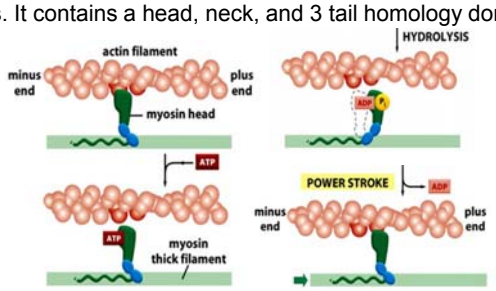
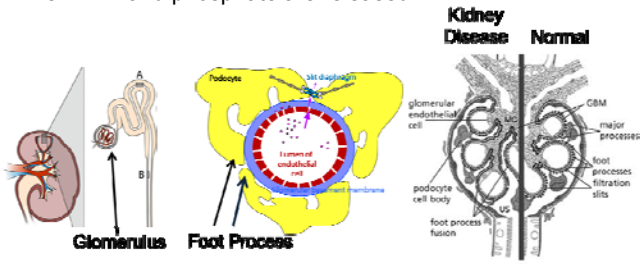


Figure 16-61 from Alberts et al. Molecular Biology of the Cell 2008.

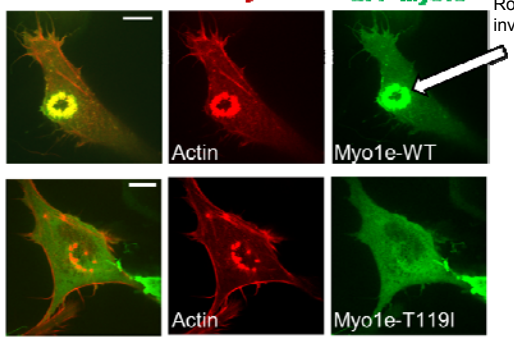
Myosin moves along actin filaments using the activity of its head domain. Myosin releases actin upon ATP binding, changes conformation after ATP hydrolysis , and reattaches when ADP and phosphate are released.



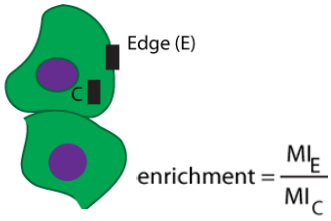
From The Association of Medical Illustrators. Serbin Communications 2013.; Figure from Bi et al. Characterization of Myosin 1e Function in Maintaining Cell Junction Integrity in the Kidney, 2012.; Figure 1A From Wang and Takezawa. J Biosci Bioeng 2005.

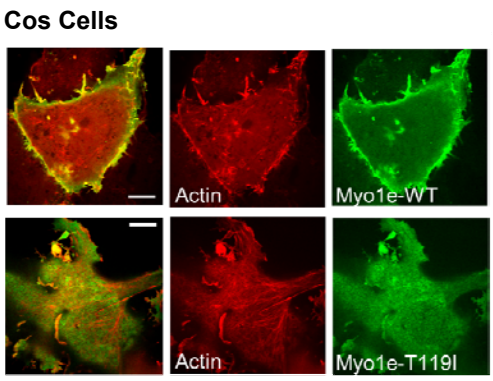
Mutations in myo1e have been shown to disrupt glomerular filtration within kidneys, leading to kidney disease (*Krendel et al., J. Am. Soc. Nephrol. 2009*). The T119I mutation was one of the myo1e mutations found to be linked to renal disease in a clinical study (*Al-Hamed et al., J. Hum. Genet. 2013*).

Results

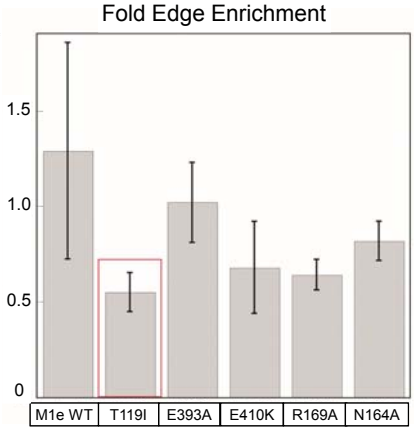


Invadopodia are specialized adhesion structures formed by cancer cells and self-organize into rosettes (*Albiges-Rizo et al., J. Cell Sci. 2009*).





Localization of Myo1e constructs relative to actin marker Lifeact within cells. Scale bars – 10 um.



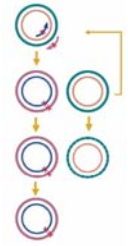
Conclusion

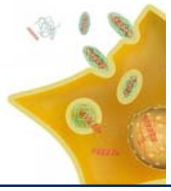
T119I mutated myo1e protein is mislocalized compared to WT myo1e. Fold edge enrichment analysis provides evidence for the mislocalization seen in images. This data indicates the T119I mutation disrupts localization of Myo1e within cells.

Methods

Introducing the T119I mutation into GFP tagged Myo1e DNA was done by site directed mutagenesis.

Figure 1 from QuikChange Lightning Site-directed Mutagenesis Kit. Agilent Technologies 2013.





Purified GFP-myo1e DNA containing the T119I mutation was transfected into cultured cells using a transfection agent (JetPEI) before imaging.

Figure from DNA Transfection Reagent JetPEI. Peqlab 2013.

Future Directions

- Analyze junctional enrichment of T119I mutated myo1e using cultured MDCK cells
- Prepare adenoviral vector encoding myo1e-T119I for expression in podocytes
- Analyze ATP binding, actin binding and motor activity of the mutants

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