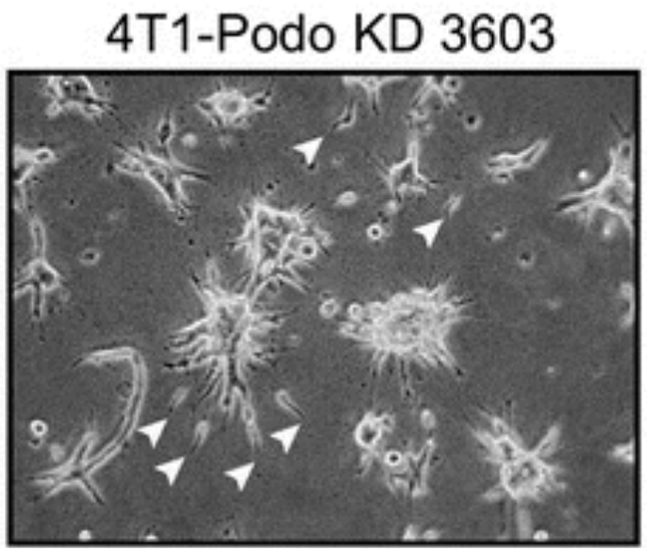
Invasion Transformation Resulting From Podocalyxin Knockdown

Tate Goodman
For: CAPS 200



Taken From: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4722710/ [2]

Invasion

- Inhibition of one invasion ‘mode’ often causes a transition to a new ‘mode’[1,2,10].

- Single-cell invasion modes are amoeboid or mesenchymal[1].

- Amoeboid invasion is dependent on Rho/ROCK based contractility[1,5,6].

- Mesenchymal invasion displays upregulated vimentin and N-cadherin, invading independent of Rho/ROCK pathways[1,6].

- Over expression of podocalyxin drives collective cell invasion[2,7].

Podocalyxin

- Podocalyxin is linked to Ezrin and NHERF.

- Podocalyxin activates RhoA[3,8].

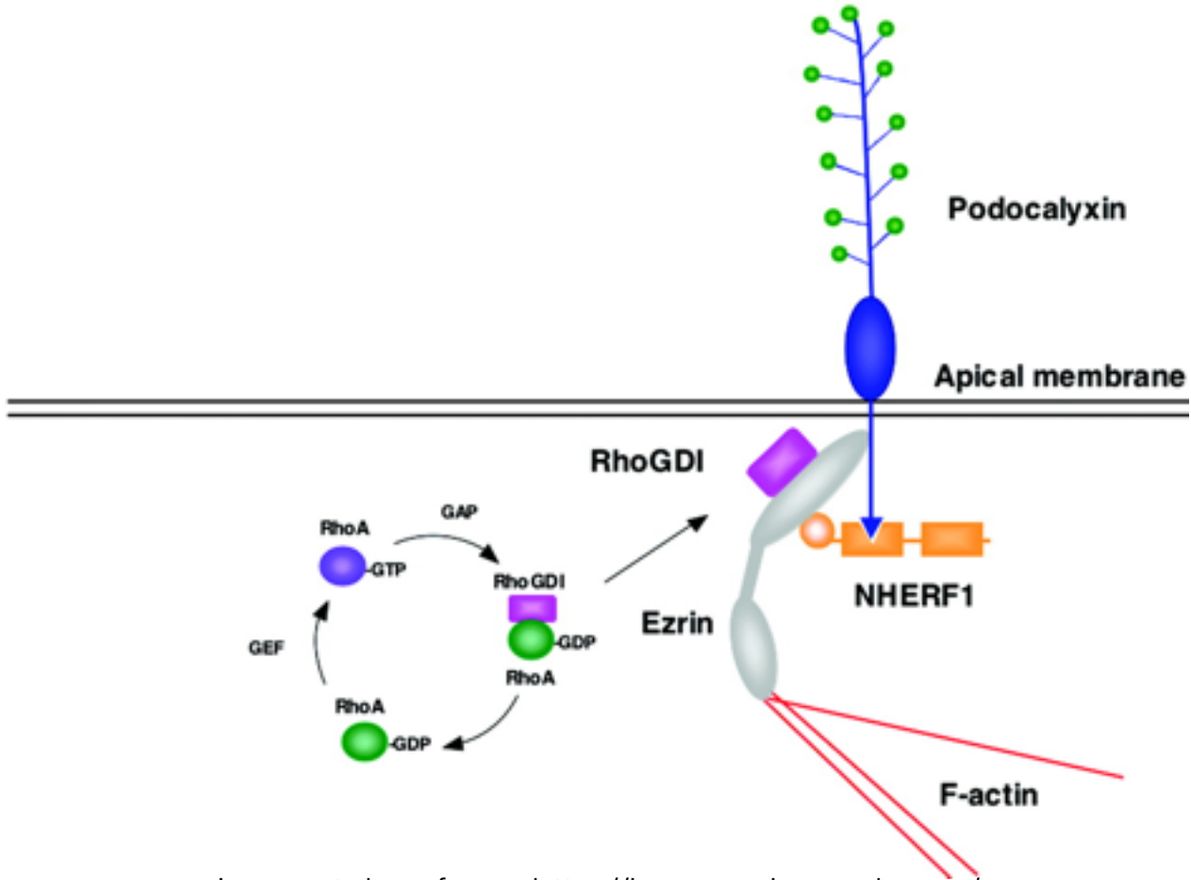


Image taken from: http://jasn.asnjournals.org/content/15/9/2289/F9.expansion [8]

- Podocalyxin expression contributes invadapodia in mesenchymal invasion[4].

- Knockdown/blocking podocalyxin blocks collective invasion through the ECM[2,7].

- 4T1 podocalyxin knockdown cells transitioned to single-cell invasion[2].

Question & Hypothesis

- How do cells invade when invasion driver podocalyxin is inhibited to block collective invasion.

- If podocalyxin is knocked down in collectively invasive cancer cell lines, EMT will be initiated as a result of decreased activated RhoA.

Specific Aims

- Classify mode of invasion.
- Determine universality.
- Find out if this is specific to knockdowns.
- Look into invasion pathways.

Classifying Invasion

- Amoeboid
    - Block ROCK1 with Y-27632 in 4T1 podocalyxin knockdown cells.
        - Observe invasion through phase contrast videos, comparing against 4T1 podocalyxin knockdown cells.
- Mesenchymal Invasion
    - Fix and stain cells for vimentin, N-cadherin and actin using confocal microscopy.

Universality & Mechanism

- Universality:
  - Knockdown podocalyxin using shRNA in MiaPaca and HT29 cells.
  - Observe invasion in 3D through phase contrast.
  - Knockout podocalyxin in 4T1, imaging in phase contrast.
- Mechanism:
  - RNA-seq looking for, SNAIL1, SNALI2/SLUG or TWIST1[9], comparing against standard 4T1 cells.
  - Active RhoA assay, comparing concentrations in 4T1 and 4T1 podo KD cells.

Expected Outcomes

- Inhibition of ROCK will have minor to no effect.
- 4T1 podocalyxin knockdown cells will invade through EMT.
- Mia PaCa, HT29 cells will display similar single-cell invasion modes.
- 4T1 podo knockout cells will display decreased single-cell invasion capacity.
- Decreased active RhoA may cause EMT.

Significance

- Podocalyxin is a clinically significant target.
- Metastasis causes majority of cancer deaths.
- Podocalyxin therapy will be ineffective if another mode of invasion is activated.
- Future directions:
  - Will single-cell invasion be initiated in vivo?
  - Mechanism of action of the transformation.
  - Can this transformation be blocked?

References

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