Interactions Between Pdlim7 and Yap1 during Trigeminal Ganglion Neurodevelopment

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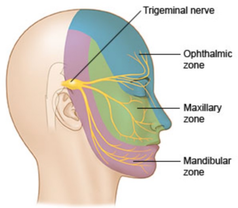
Background

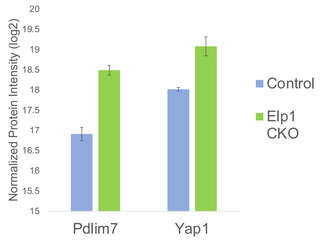
The trigeminal ganglion (TG) contains the cell bodies for neurons that relay sensations in the face, including pain, temperature, and touch.



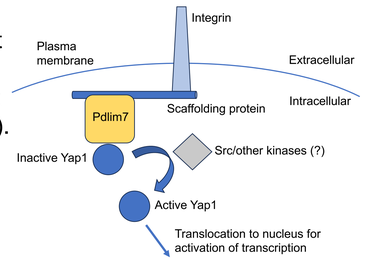
From https://livingwiththeal.com/what-is-trigeminal-neuralgia/

In the genetic disorder familial dysautonomia (FD), mutations in *ELP1* reduce ELP1 protein levels and cause neuronal dysfunction, leading to abnormal TG development and sensory deficits (Anderson et al., 2001).

Preliminary data from a mouse model of FD suggest that loss of Elp1 increases expression of Pdlim7 and several proteins in the Yap1 signaling pathway in the TG.



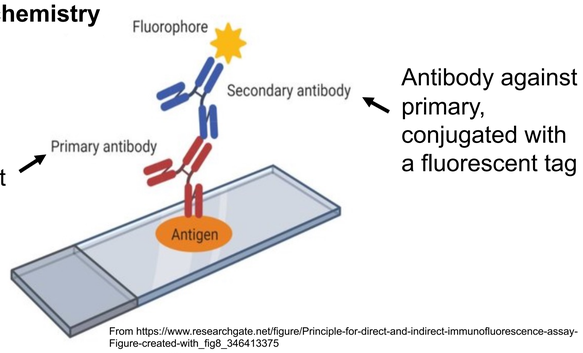
In other contexts, Pdlim7 facilitates activation of Yap1 through binding, but the role of Pdlim7, and its interaction with Yap1 in TG development, has not been described (Elbediwy et al., 2018).



Hypothesis: Pdlim7 and Yap1 physically interact in the TG during embryonic development.

Methods

1. Immunohistochemistry

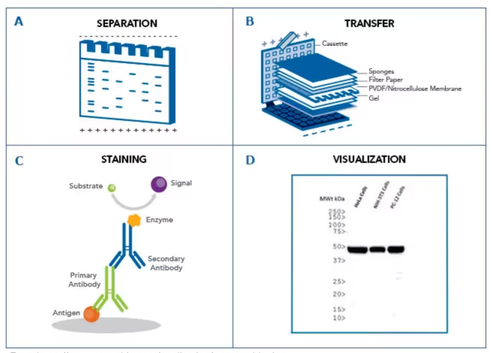


From https://www.researchgate.net/figure/Principle-for-direct-and-indirect-immunofluorescence-assay-Figure-created-with_fig8_346413375

2. Immunoblotting

Separation of proteins by size →

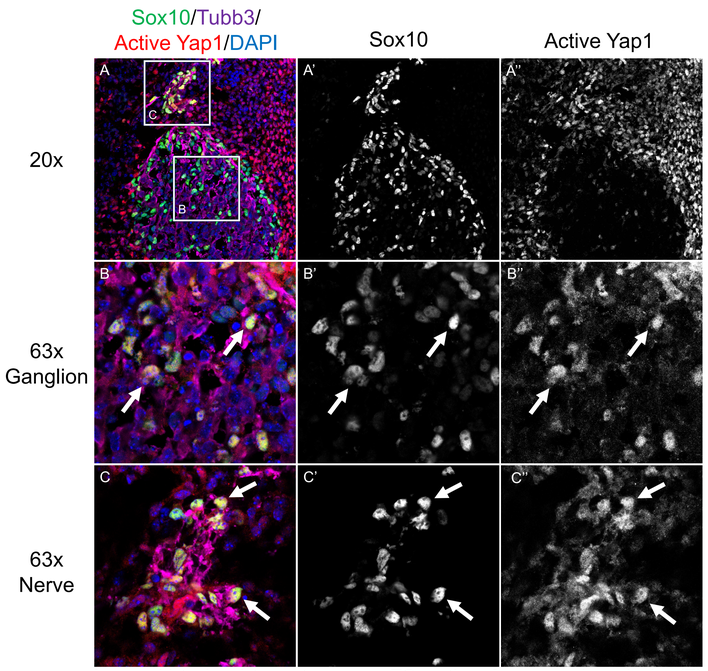
Specific detection of target protein using antibodies →



From https://www.novusbio.com/application/western-blotting

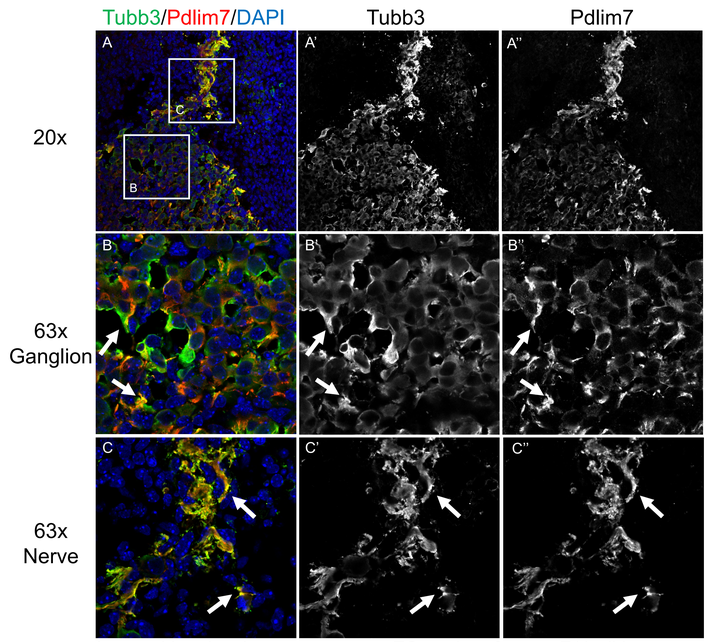
Results

1. Active Yap1 co-localizes with Sox10, which marks nuclei of non-neuronal precursor cells.



Immunohistochemistry on transverse TG sections from an embryonic day (E) 11 wildtype mouse at 20x (A-A''). Boxes in (A) show regions at higher magnification in (B-B'', C-C''), with Sox10 or Active Yap1 in white (A', A'', B', B'', C', C''). Arrows point to co-localization of Active Yap1 and Sox10 in the nuclei of non-neuronal cells.

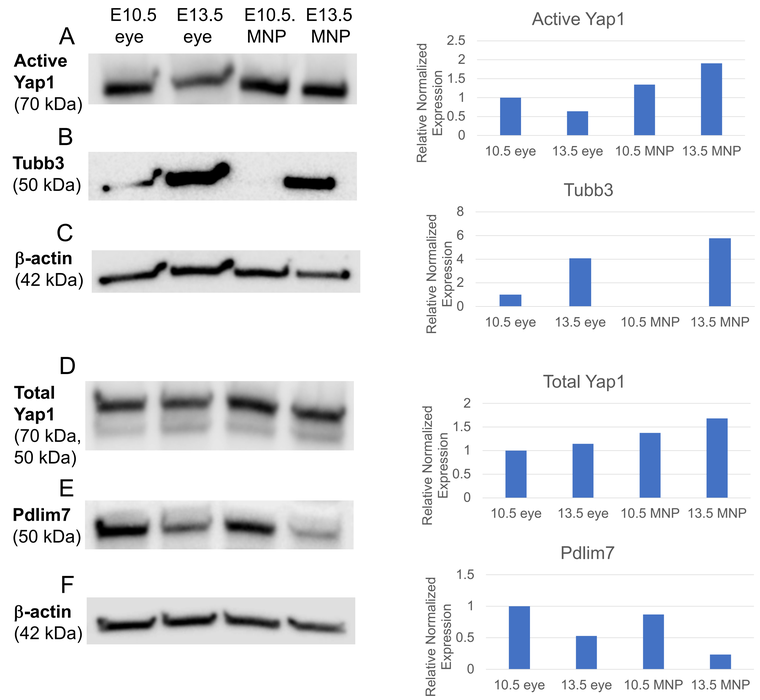
2. Pdlim7 is enriched in the cytoplasm of Tubb3-positive neurons.



Immunohistochemistry on transverse TG sections from an E11 wildtype mouse at 20x (A-A''). Boxes in (A) show regions at higher magnification in (B-B'', C-C''), with Tubb3 or Pdlim7 in white (A', A'', B', B'', C', C''). Arrows point to co-localization of Pdlim7 and Tubb3 in the cytoplasm of neurons.

Results

3. Yap1 and Pdlim7 are dynamically expressed in trigeminal nerve target tissue containing axons of TG neurons from E10.5 to E13.5.



Immunoblotting carried out on whole-cell lysates prepared from the eye/frontal nerve region (eye) and the mandibular region (MNP), which contain axons of TG neurons, of E10.5 and E13.5 wildtype mice. (A) Active Yap1, (B) Tubb3, (D) Total Yap1, (E) Pdlim7, and (C, F) β-actin (control) for Active Yap1 and Tubb3, or Total Yap1 and Pdlim7, respectively (left). Total Yap1 recognizes both Active (top, 70 kDa) and Inactive (bottom, 50 kDa) Yap1. Band intensities for Active Yap1, Tubb3, Total Yap1 (combined Active and Inactive), Pdlim7, and each β-actin blot were quantified using ImageJ software. Relative protein levels were ascertained by normalizing each protein volume to its respective β-actin volume and dividing by the normalized E10.5 eye sample, setting the value for this sample to one (right).

Conclusions and Future Directions

Conclusions

- Active Yap1 is enriched in non-neuronal precursor cells, which include neural crest cell precursors. Pdlim7 is expressed in the cytoplasm of early TG neurons.
- Pdlim7 expression and Yap1 activity change throughout embryonic development as projections from TG neurons invade target regions.
- Dysregulation of Pdlim7 and Yap1 may contribute to neurodevelopmental phenotypes observed in FD.

Future Directions

- Determine whether Pdlim7 facilitates Yap1 activation via direct binding in TG neurons
- Confirm whether Pdlim7 and Yap1 distribution and expression are disrupted in TG of the FD mouse model, and examine how this dysregulation alters TG neurodevelopment

Acknowledgements

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