Investigating the Roles of Striatin in Tight Junctions

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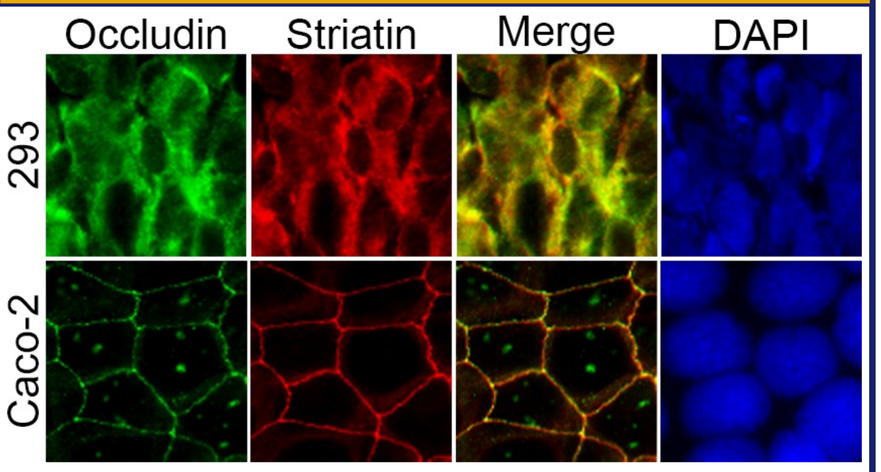
Introduction

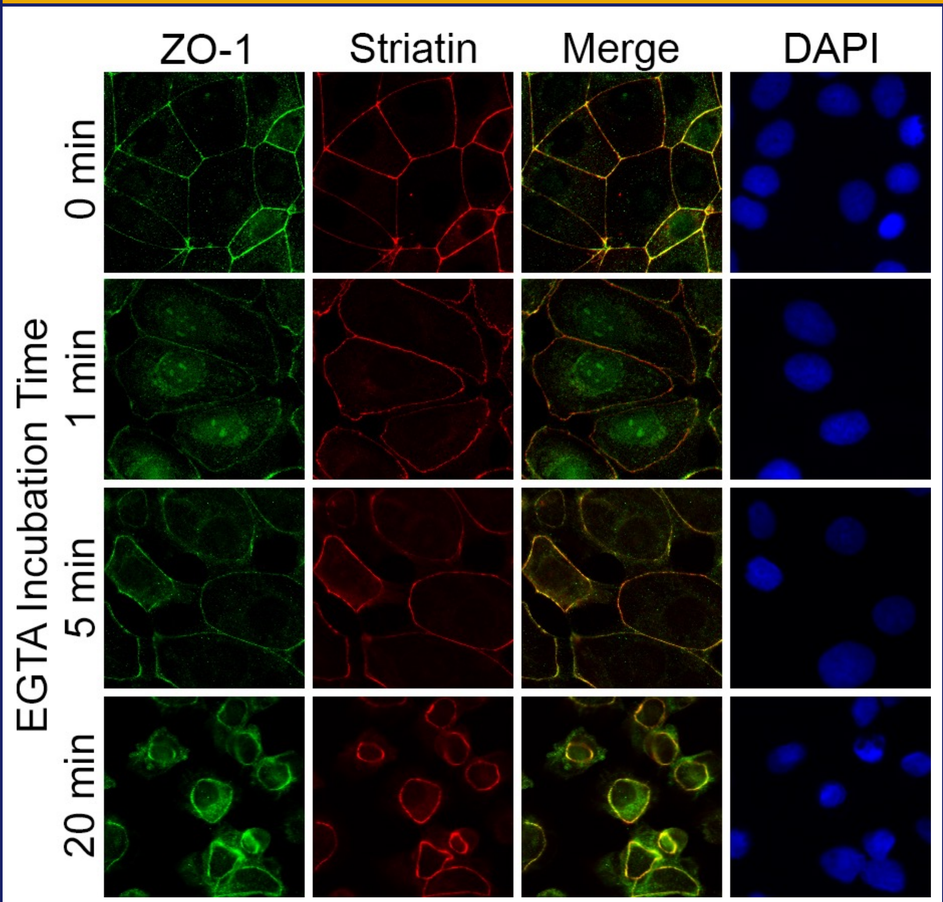
- Epithelium lines the outer surfaces of hollow organs and blood vessels and plays important physiological roles of protection and regulation.
- Tight junctions (TJs) form the continuous intercellular barrier within epithelium and are integral to their functions, whose dysfunctions associate with pathologies such as cancer.
- Striatin and striatin-interacting and phosphatase and kinase (STRIPAK) is the regulatory B subunit of protein phosphatase 2 (PP2A), and they have been reported to regulate essential cell processes of vesicle transport and cell polarity.
- This project strives to unravel the roles and mechanisms of striatin and STRIPAK in the functions and regulations of TJs.

Methods & Results

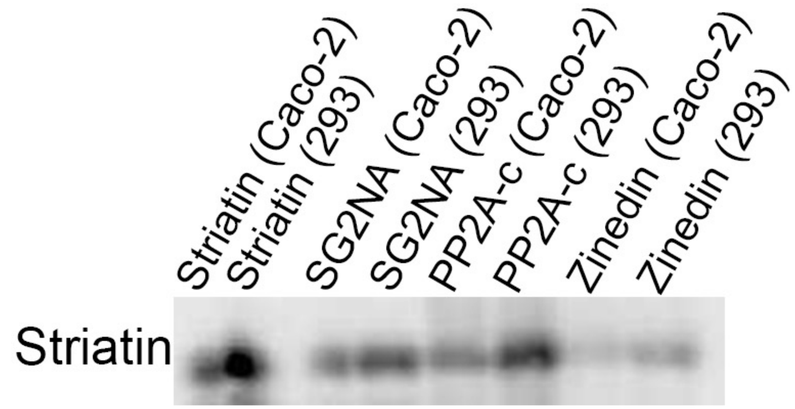


▲ **Figure 1. Striatin is co-localised with occludin in TJ.** Caco-2 and QBI 293 cells were fixed in 4% PFA, permeabilised with 0.1% Triton X-100 in PBS, treated with aqueous solution containing 1.3% SDS and 0.52% DTT prior to blocking, and stained for the presence of occludin and striatin. Additional experiments also found co-localisation with occludin, claudin and cingulin (results not shown).

Methods & Results (Cont'd)



◂ **Figure 2. Striatin is co-localised with TJ proteins during TJ disassembly.** Caco-2 cells were cultured in low Ca^{2+} media for 12 hours. Then, cells were incubated with EGTA for 1, 5, and 20 mins prior to being fixed in in 4% PFA, permeabilised with 0.1% Triton X-100 in PBS and stained for the presence of ZO-1 and striatin. When TJs separate as induced by EGTA, striatin colocalises with TJ protein, ZO-1.



▲ **Figure 3. Striatin interacts with PP2A subunits and TJ proteins.** Lysed Caco-2 and 293 cells were immunoprecipitated with antibodies as shown in the figure, analysed with 1D SDS-PAGE and blotted with striatin monoclonal antibody.

Discussions

- Preliminary data from IF, IP, and Western Blots showed that striatin is localised in TJs with TJ proteins, such as occluding, ZO-1, etc. during normal physiology and TJ breakdown. As the regulatory B subunit of PP2A, striatin also interacts with other members of TJ and PP2A.
- Further experiments will test:
  - localisation of striatin during TJ assembly after breakdown;
  - interactions of other STRIPAK members, such as CCM-3 and Mob;
  - TJ disassembly and assembly after using lentivirus to generate Caco-2 cell lines stably expressing striatin and its mutants.

Acknowledgements

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