Cortactin Scaffolds Arp2/3 and WAVE2 at the Epithelial Zonula Adherens

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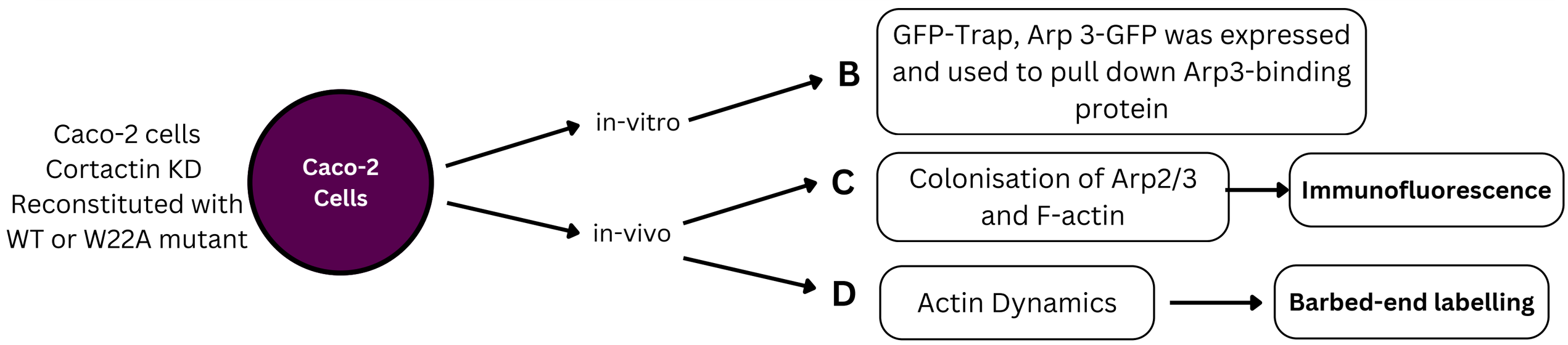
BACKGROUND

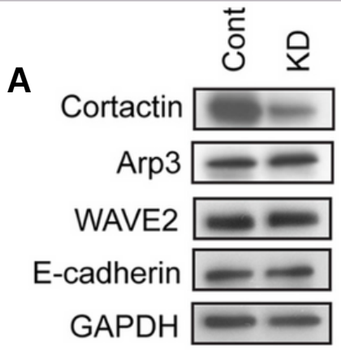
The epithelial zonula adherens (ZA) is a specialized cell-cell adhesive junction that integrates E-cadherin-mediated adhesion and the actomyosin cytoskeleton to maintain epithelial organization and junctional tension. Actin assembly at the ZA is regulated by the Arp2/3 complex, which requires co-factors like WAVE2 for nucleation and N-WASP for filament stabilization. E-cadherin plays a key role in recruiting Arp2/3 and WAVE2 to the ZA, marking sites for actin assembly. However, these interactions depend on active cadherin adhesion.

Cortactin, a multidomain scaffolding protein, is proposed to coordinate actin nucleation at the ZA by binding and activating Arp2/3, interacting with WAVE2, N-WASP, and stabilizing branched actin networks. This study highlights cortactin's role in spatially and temporally integrating actin regulators, ensuring precise actin assembly at the ZA to support junctional integrity and epithelial function.

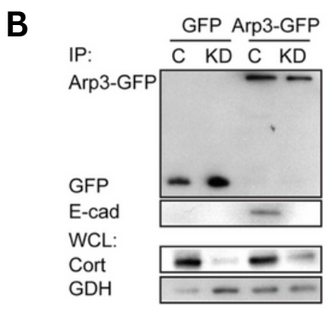
METHODS



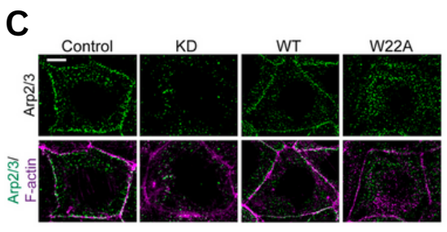
RESULTS AND ANALYSIS



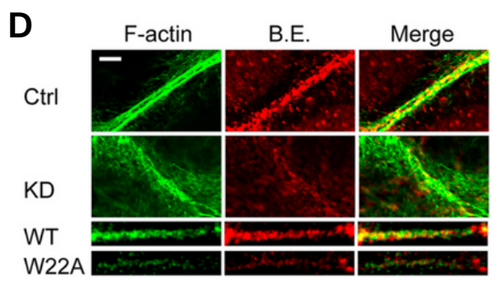
A: Western analysis confirmed cortactin knockdown (KD) in cell lysates. Arp3, Wave2 and E-cadherin levels were assessed with GAPDH as a loading control.



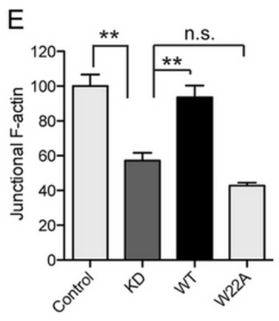
B: Arp3-GFP immunoprecipitated E-cadherin in control cells but not in cortactin KD cells.

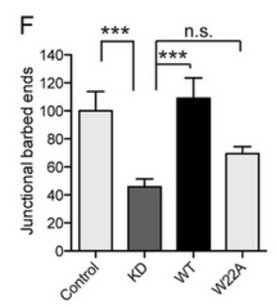


C: Reduced junctional Arp3 in cortactin KD cells as compared to the control. Wild type cortactin restored Arp3 localisation at junctions whereas the W22A mutant, incapable of binding Arp2/3 did not prove to do so.



D: Intercellular contacts showed reduced barbed ends and dispersed F-actin in cortactin KD cells. WT cortactin restored F-actin and barbed end localisation at the Zonula adherent (ZA) but W22A did not.





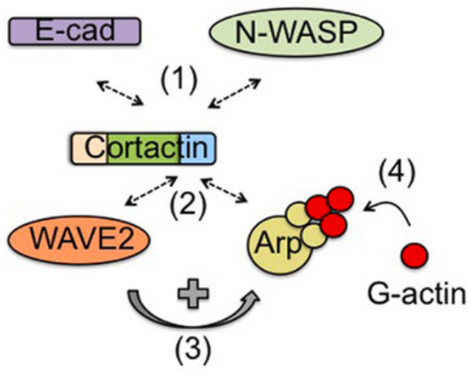
E and F: Quantitative analysis revealed significant difference in junctional F-actin and barbed end labelling between the the control, KD cortactin, WT and W22A. WT cortactin managed to restore normal actin structural while W22A did not manage to do so.

DISCUSSION

This study postulates cortactin as a critical scaffold at the epithelial zonula adherens (ZA), integrating signals from E-cadherin and N-WASP to regulate actin assembly. Cortactin localizes specifically to apical junctions and is necessary for recruiting both Arp2/3 and WAVE2. Loss of cortactin reduced junctional Arp2/3 levels and disrupted actin nucleation, shown by diminished barbed-end labeling and F-actin content

These results highlight cortactin's dual scaffolding role, independently coordinating Arp2/3 and WAVE2, ensuring spatial precision and actin polymerization at the ZA. Its recruitment depends on E-cadherin and N-WASP, illustrating how adhesion molecules and actin regulators stabilize the cytoskeleton.

These findings enhances our understanding of cadherin-actin integration in maintaining epithelial integrity and junctional tension.



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