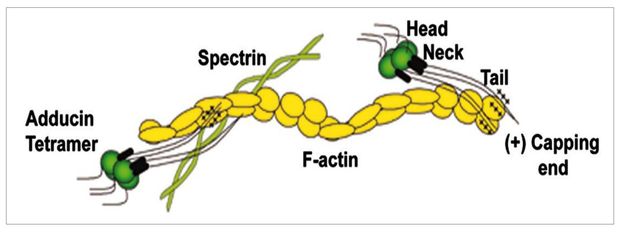
Adducin modulates intercellular keratinocyte adhesion

Vera Rötzer, Jens Waschke, Volker Spindler
Institute of Anatomy and Cell Biology, Department I, LMU Munich, Pettenkoferstraße 11, D-80336 Munich

Introduction

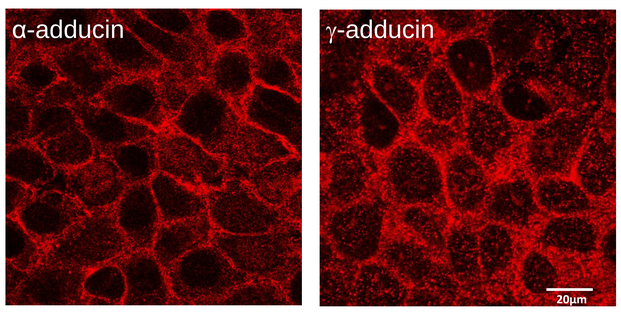


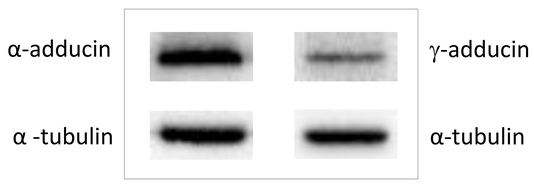
modified from Pariser et al, PNAS, 2005

Adducin is an ubiquitously expressed protein of the actin-based membrane cytoskeleton. It is located at the spectrin-actin network thereby recruiting spectrin to fast-growing ends of actin filaments and capping the ends with high affinity in the presence of spectrin. Regulation of the membrane cytoskeleton by adducin is well established and recent data also suggest a role for intercellular adhesion.

Furthermore, since loss of keratinocyte cohesion induced by autoantibodies in the blistering skin disease pemphigus vulgaris is paralleled by pronounced actin reorganization, we studied the role of adducin in intercellular adhesion.

Adducin isoforms α and γ are both present in confluent human keratinocytes (HaCaT)

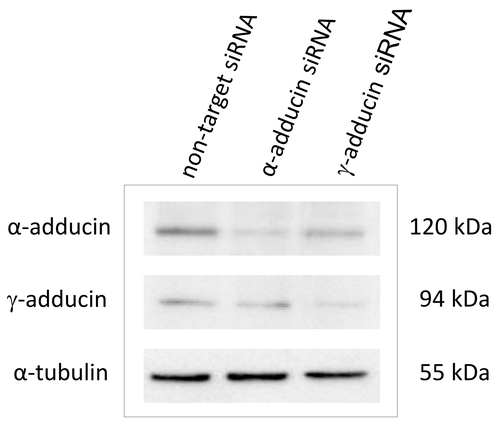


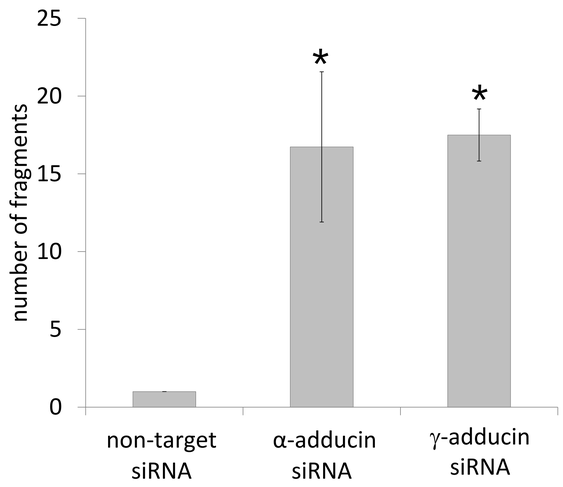


Silencing of α- or γ -adducin results in loss of keratinocyte cohesion

To investigate the role of adducin in HaCaT cells, siRNA-mediated gene silencing was performed. In controls, cells were transfected with non-targeting siRNA.

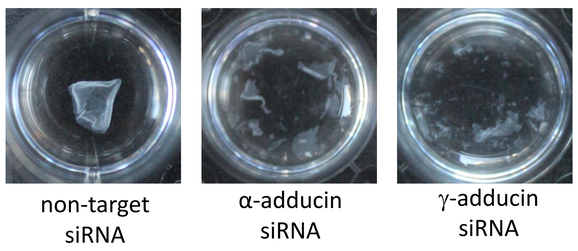
Western Blot analysis of HaCaT cell lysates demonstrated successful knockdown of either α- or γ-adducin.



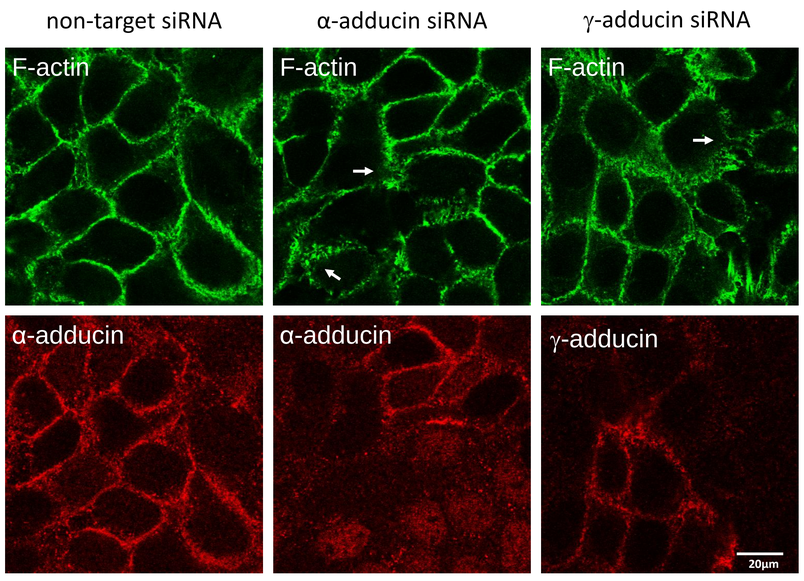


n = 5, * p < 0.05

To quantify intercellular adhesion, a dispase-based assay was applied.
48 h after knockdown of either α- or γ-adducin, cell monolayers were released from well bottoms using dispase, exposed to mechanical stress, and resulting fragments were counted.
siRNA-mediated silencing of either adducin isoform decreased intercellular adhesion compared to control knockdown.

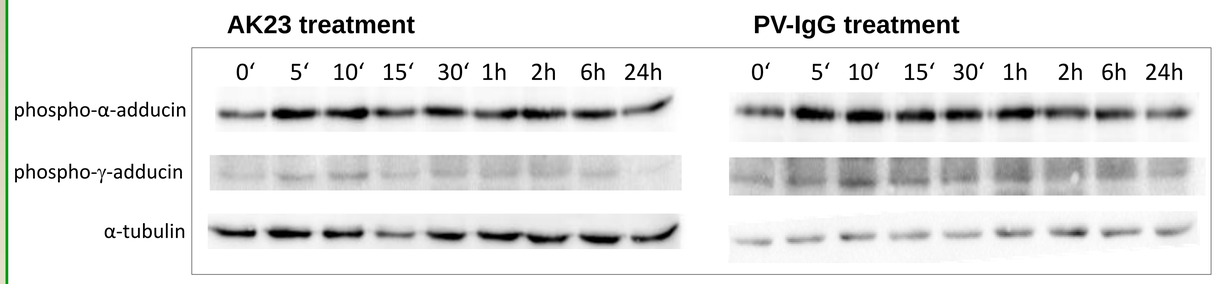


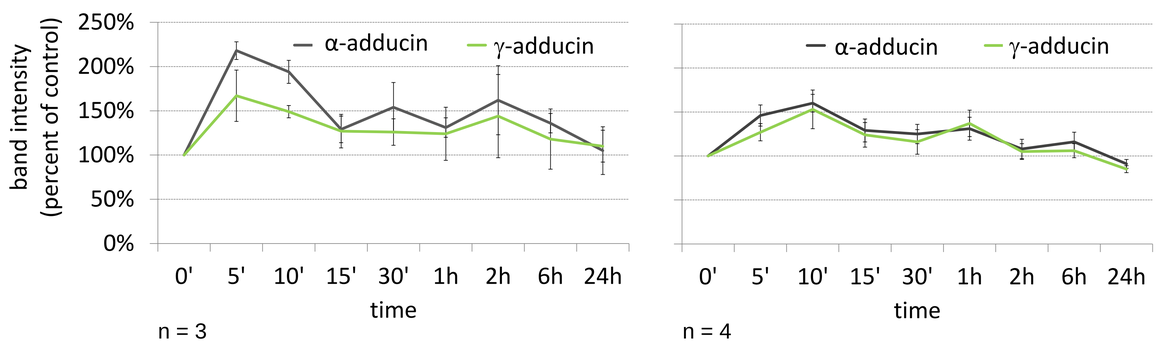
Cortical actin filaments appeared disorganized in cells with effective adducin silencing, as demonstrated by phalloidin staining.



Pemphigus autoantibodies induce rapid phosphorylation of both adducin isoforms

Incubation with AK23, a monoclonal pathogenic antibody derived from a pemphigus mouse model or with IgG fractions from pemphigus vulgaris patients (PV-IgG), both targeting the desmosomal adhesion molecule desmoglein 3, resulted in adducin phosphorylation at Serin726 starting after 5 minutes.

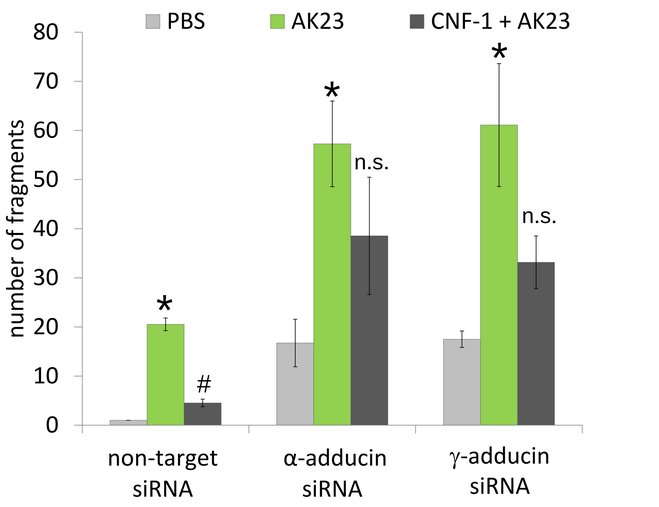




The protective effect of RhoGTPase activation on loss of cell adhesion is ameliorated by adducin silencing

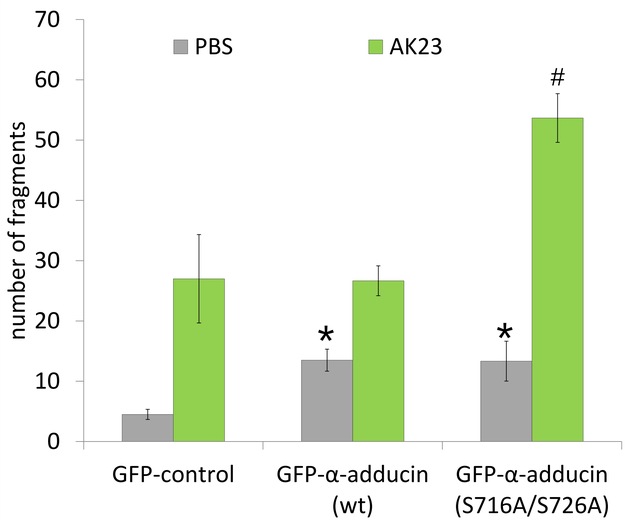
In cells transfected with non-targeting siRNA, incubation with AK23 induced loss of cell-cell adhesion after 24h. Activation of RhoGTPases RhoA, Rac1 and Cdc42 by *E.coli* cytotoxic necrotizing factor 1 (CNF-1), abrogated the effect of AK23 in these cells.

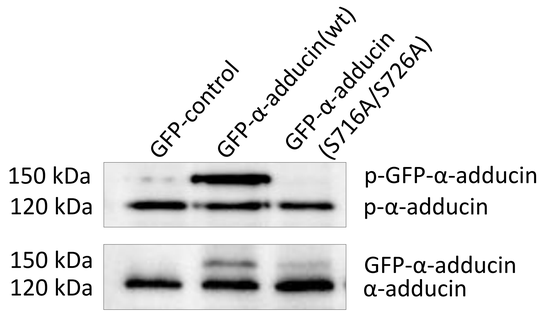
Under conditions of α- or γ-adducin silencing, the protective effect of CNF-1 was ameliorated compared to cells transfected with non-targeting siRNA.



n = 5; p < 0.05 * vs. controls; # vs. AK23

Interference with adducin phosphorylation alters cell adhesion





n = 6; p < 0.05 * vs. control/PBS; # vs. control/AK23

After transient expression of GFP-α-adducin (wt) or its phosphorylation-deficient mutant GFP-α-adducin (S716A/S726A), cell-cell adhesion was measured by dispase-based dissociation assays.
Under control conditions, the number of fragments increased after transfection of both α-adducin wt and mut, compared to controls.
Incubation with AK23 for 24 hours further increased cell dissociation only in GFP-α-adducin (S716A/S726A)-expressing cells.

Conclusions

- These experiments demonstrate that adducin is necessary for proper keratinocyte intercellular adhesion.
- Since AK23 or PV-IgG induce phosphorylation of adducins and the phosphorylation state of adducins alters cell adhesion, an involvement in the pathogenesis of pemphigus vulgaris is possible.

→In view of our preliminary results showing adducin phosphorylation after CNF-1 treatment, adducin phosphorylation seems to be a protective keratinocyte response to pemphigus autoantibody challenge.