Heterophilic Dsg3-Dsg2 interactions as a compensatory mechanism in pemphigus

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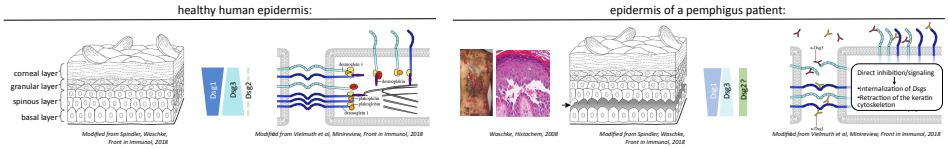
Introduction: desmosomes, epidermis and pemphigus



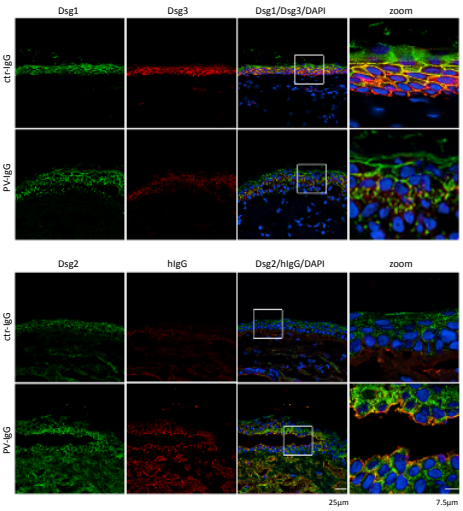
The epidermis is a multilayered tissue which provides physical barrier and protection. In the autoimmune disease pemphigus vulgaris autoantibodies against Desmoglein (Dsg)1 and 3 cause loss of intercellular adhesion clinically manifested by blistering in the suprabasal layer of the epidermis (arrow) and the mucous membranes especially of the oral cavity.

Dsg1 and 3 are desmosomal cadherins which maintain strong intercellular adhesion of desmosomes via their extracellular domains. In the epidermis they depict a differentiation- and thus layer specific expression pattern.

Interestingly, Dsg2 is almost absent in healthy human epidermis but was reported to be upregulated in pemphigus.

Aim:
Analyze altered Dsg2 expression and the impact of the heterophilic Dsg3-Dsg2 interactions in pemphigus

Pemphigus autoantibodies induce upregulation of Dsg2 in human epidermis



Immunofluorescence staining of *ex vivo* human epidermis derived from body donors showed different expression pattern of Dsg1 and 3 in samples injected with IgG-fractions of healthy volunteers (ctr-IgG). Opposing gradients reveal that Dsg1 was predominantly located in superficial layers whereas Dsg3 was more abundant in basal layers.

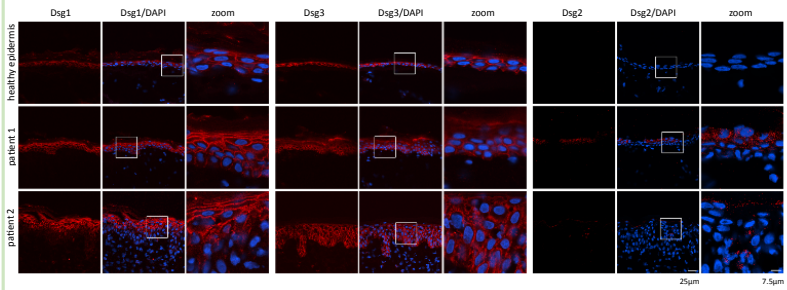
Injection with an IgG-fraction of a pemphigus vulgaris patient (PV-IgG) induced suprabasal blister formation accompanied with reduced and fragmented epidermal Dsg1 and 3 staining (Dsg depletion), respectively.

Interestingly, Dsg2 was almost absent in ctr-IgG injected samples. PV-IgG injection induced increased levels of Dsg2, particularly at the membranes of keratinocytes which surrounding the blister.

An antibody directed against the Fc-part of human IgGs (hIgG) was used to detect PV-IgGs bound to epidermal keratinocytes and confirmed that no ctr-IgGs were bound.

Representatives of N=3

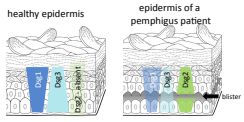
Dsg2 is upregulated in pemphigus patients



Immunofluorescence staining of epidermis of pemphigus patient 1 showed normal Dsg1 staining whereas staining was fragmented in patient 2. Dsg3 of both patients was depleted. Interestingly 3 out of 6 patients had increased levels of Dsg2. Dsg2 in patient 1 was highly upregulated with a spotted localization mainly in superficial layer, while patient 2 had less Dsg2 but also in deeper layers.

Representatives of 6 patients

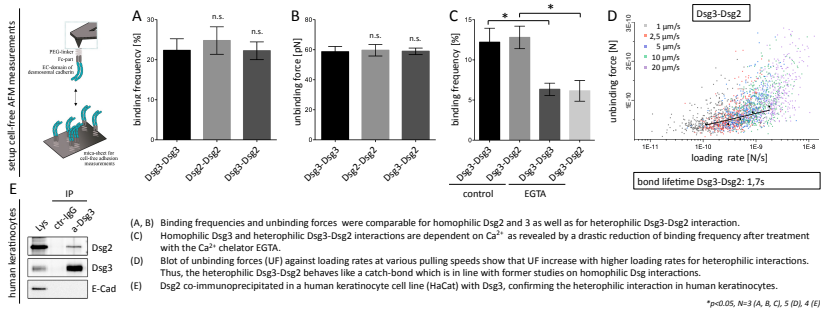
Summary I



Dsg2 was upregulated in:
- *ex vivo* human pemphigus model
- pemphigus patients

Question:
Function and mechanism of Dsg2 upregulation?

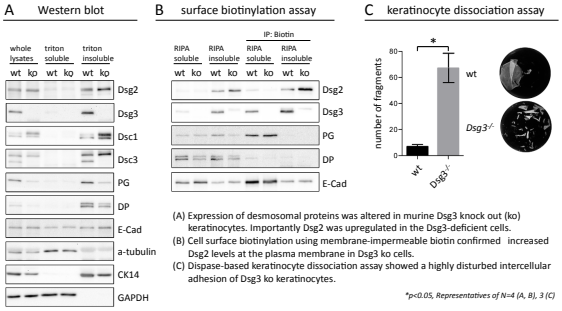
Heterophilic Dsg3-Dsg2 interactions behave similar to homophilic Dsg interactions



(A, B) Binding frequencies and unbinding forces were comparable for homophilic Dsg2 and 3 as well as for heterophilic Dsg3-Dsg2 interaction.
(C) Homophilic Dsg3 and heterophilic Dsg3-Dsg2 interactions are dependent on Ca^{2+} as revealed by a drastic reduction of binding frequency after treatment with the Ca^{2+} chelator EGTA.
(D) Blot of unbinding forces (UF) against loading rates at various pulling speeds show that UF increase with higher loading rates for heterophilic interactions. Thus, the heterophilic Dsg3-Dsg2 behaves like a catch-bond which is in line with former studies on homophilic Dsg interactions.
(E) Dsg2 co-immunoprecipitated in a human keratinocyte cell line (HaCat) with Dsg3, confirming the heterophilic interaction in human keratinocytes.

**p<0.05, N=3 (A, B, C), 5 (D), 4 (E)*

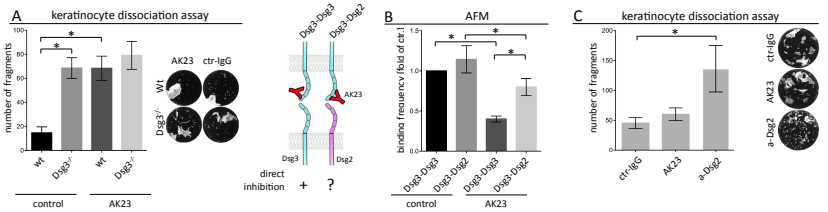
Dsg3 knock out keratinocytes show altered expression pattern of desmosomal proteins and disturbed intracellular cohesion



(A) Expression of desmosomal proteins was altered in murine Dsg3 knock out (ko) keratinocytes. Importantly Dsg2 was upregulated in the Dsg3-deficient cells.
(B) Cell surface biotinylation using membrane-impermeable biotin confirmed increased Dsg2 levels at the plasma membrane in Dsg3 ko cells.
(C) Dispase-based keratinocyte dissociation assay showed a highly disturbed intercellular adhesion of Dsg3 ko keratinocytes.

**p<0.05, Representatives of N=4 (A, B), 3 (C)*

Dsg3-Dsg2 interaction is less susceptible to a pathogenic Dsg3 antibody



(A) A pathogenic Dsg3 antibody derived from a pemphigus mouse model (AK23) led to drastically reduced intercellular adhesion in wt keratinocytes whereas no effect was observed in Dsg3-deficient keratinocytes.
(B) AK23 drastically reduced binding frequency of Dsg3 homophilic interactions whereas effect on heterophilic interactions to Dsg2 was less pronounced.
(C) Whereas AK23 had no effect on Dsg3 ko keratinocytes an anti-Dsg2 (a-Dsg2) antibody drastically reduced intercellular adhesion.

**p<0.05, N=3 (A), 5 (B), 4 (C)*

Summary II and Conclusion

- Dsg3 ko keratinocytes show an upregulation of Dsg2
- Dsg3 and 2 interact heterophilically
- Heterophilic Dsg3-Dsg2 interaction was less susceptible to AK23
- a-Dsg2 antibody disrupted intercellular cohesion of Dsg3 ko keratinocytes

► **Autoantibody induced upregulation of Dsg2 could serve as a compensatory mechanism in pemphigus**