Loss of Desmocollin 1 alters proliferation and differentiation in reconstructed human epidermis

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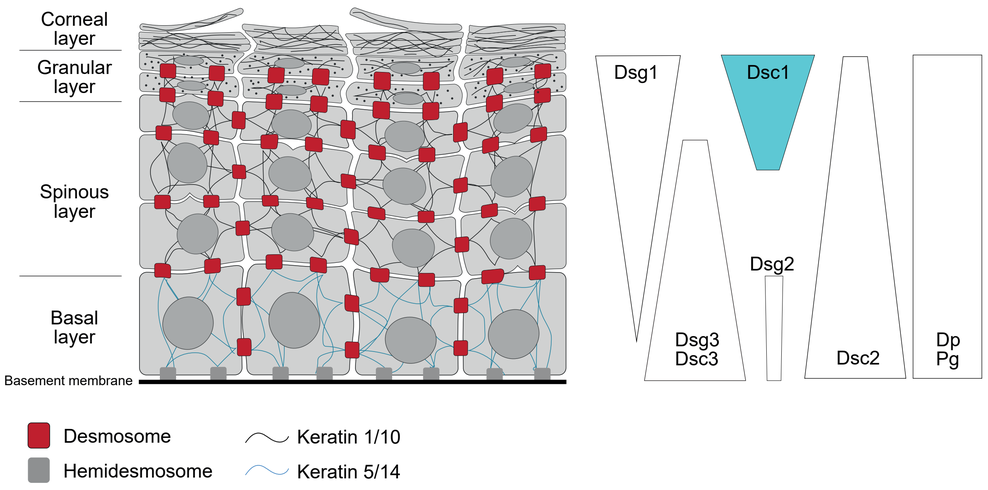
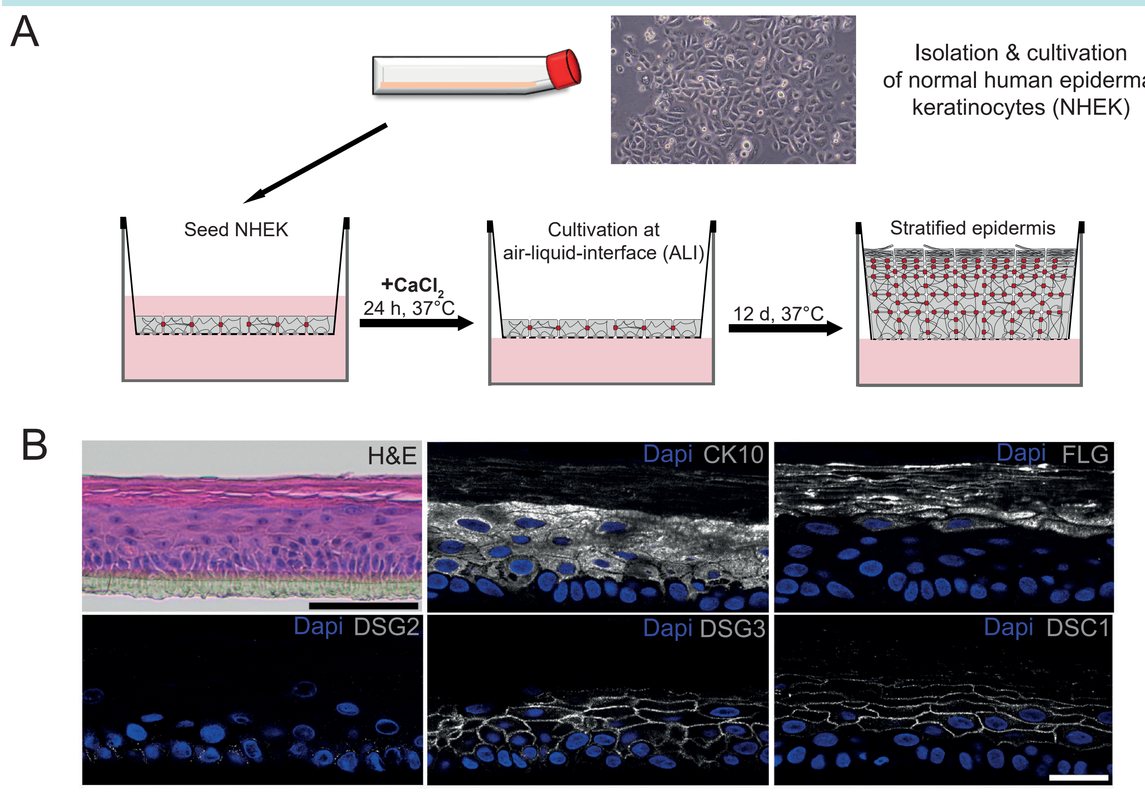
Introduction:
Desmosomes in the human epidermis



Desmosomes are not only static structures facilitating robust cell-cell-adhesion, but are also proposed to act as sensors and mediators of tissue-specific cell behaviour and signalling. The isoform-specific patterning of desmosomal cadherins, desmoglein (DSG) 1-4 and desmocollin (DSC) 1-3, within the differentiated layers of the human epidermis suggests a contribution of desmosomal cadherins to epidermal differentiation which is only partially understood so far. Desmosomal cadherins specifically expressed in the suprabasal layers are interesting with respect to terminal differentiation and barrier formation in stratified epithelia.

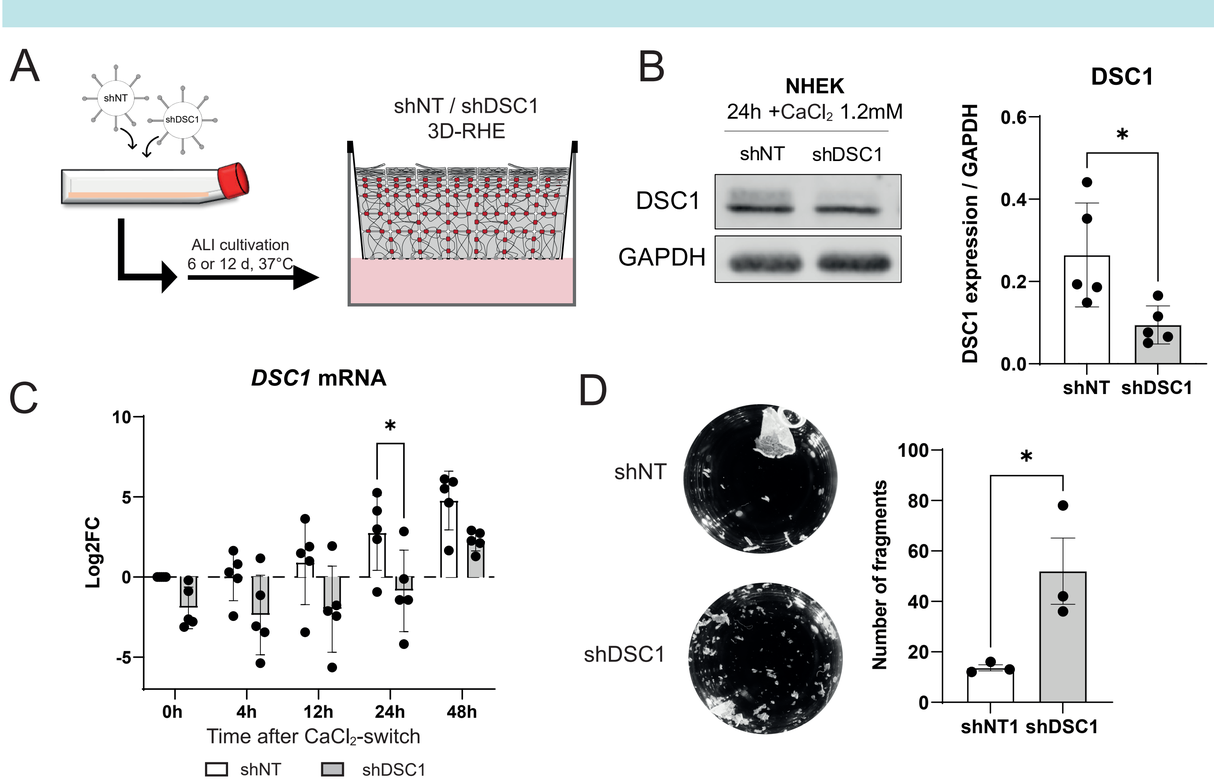
Aim: To investigate the influence of the desmosomal adhesion molecule DSC1 on the epidermal differentiation in a 3D reconstructed human epidermis (3D-RHE).

3D-RHEs mimic interfollicular epidermis



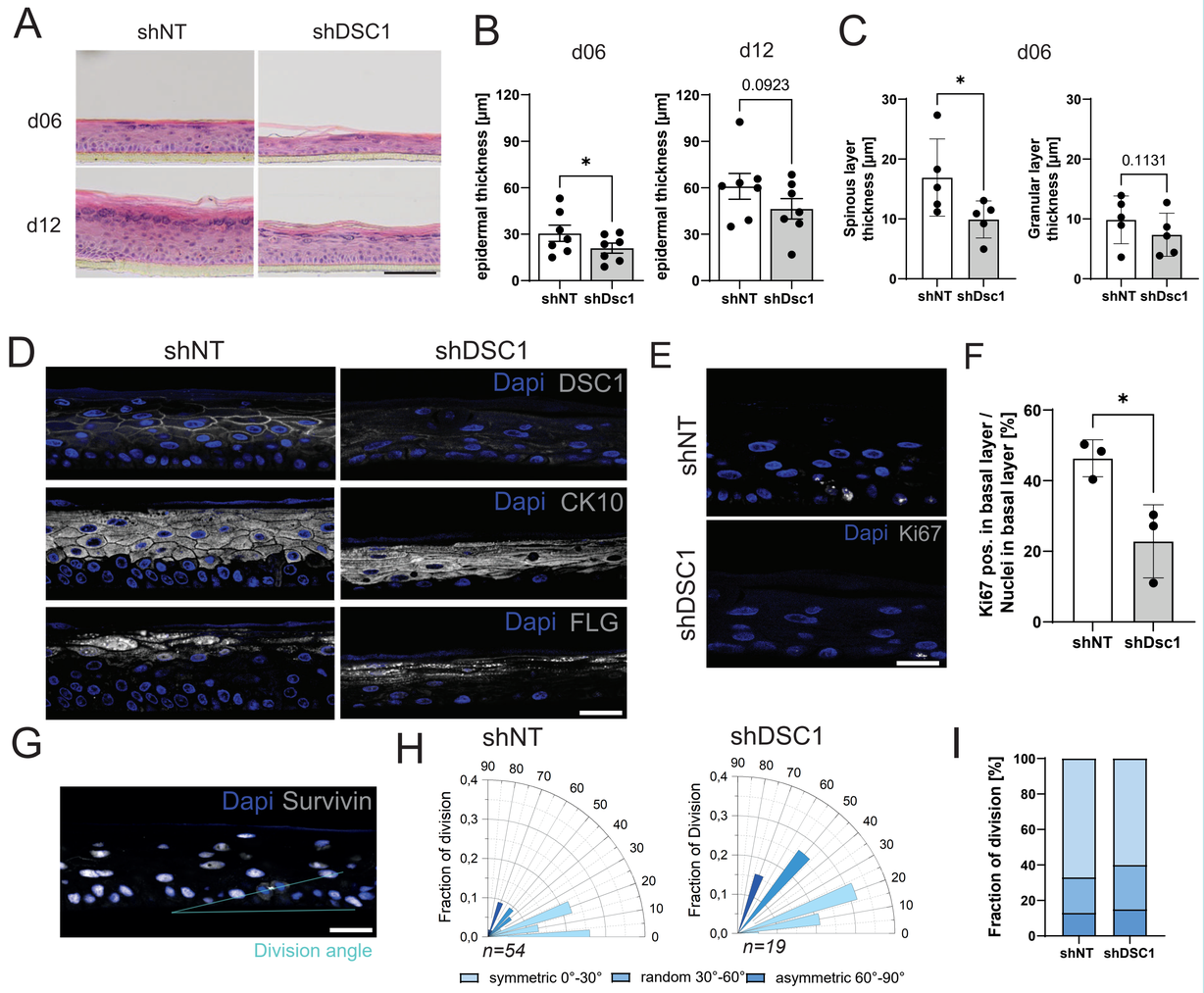
A) Principle of 3D-RHE generation. **B**) After 12 days, 3D-RHEs were fully stratified (see H&E staining), confirmed by immunostainings for the epidermal differentiation markers Cytokeratin 10 (CK10) and Filaggrin (FLG). Desmosomal cadherins were expressed similarly to human epidermis. Scale bar: 100 µm (black); 20 µm (white).

Silencing of DSC1 causes loss of cell-cell adhesion



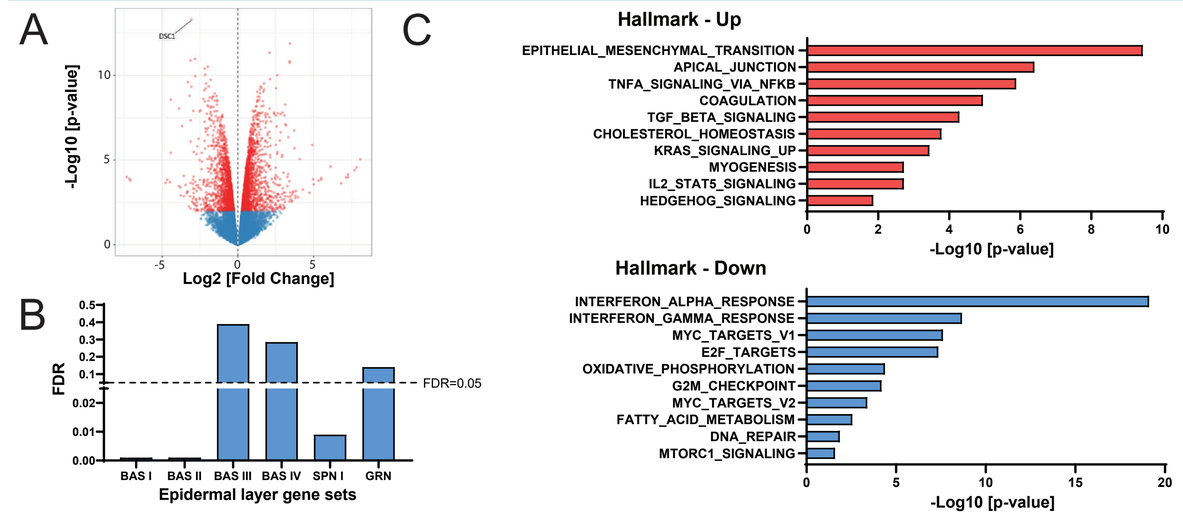
A) Scheme of 3D-RHE cultivation including DSC1 knock-down by short hairpin RNA (shDSC1; shNT as control) by lentiviral transduction of NHEKs. **B**) Western blot and quantification of DSC1 protein expression in NHEKS 24h after 1.2 mM $CaCl_2$ treatment (n=5) show silencing of DSC1. **C)** *DSC1* mRNA levels detected by qRT-PCR show an increase over time after $CaCl_2$-switch of NHEKs, significantly lower in shDSC1 keratinocytes (n=5). **D)** Knock-down of DSC1 causes loss of cell-cell adhesion 48h after 1.2 mM $CaCl_2$-switch (n=3) in a dispase-based dissociation assay.

Reduced thickness correlates with altered proliferation in shDSC1 3D-RHE



A) H&E images of 3D-RHE under shNT and shDSC1 conditions after 6 and 12 days of cultivation. shDSC1 3D-RHEs are thinner. **B**) Quantification of total epidermal thickness, showing a significant difference after 6 days of cultiation and a tendency for reduced thickness after 12 days (n=7). **C**) Analysis of spinous and granular layer thickness after 6 days (n=5) shows that reduced thickness results from a thinner spinous layer in DSC1 knock-down rafts. **D**) Immunostainings of 3D-RHE after 6 days do not show pronounced changes in differentiation markers. **E)** Immunostainings and **F)** quantification of basal expression of the proliferation marker Ki67 (n=3) suggest a reduced proliferation in shDSC1 rafts as cause for reduced epidermal thickness. **G)** Scheme of the analysis of the division anlge (light blue) with survivin for detection of the spindle midbody orientation during epidermal differentiation of keratinocytes in 3D-RHEs. **H)** Radial hisogram of cell division angles and fraction of division. Number of survivin-positive cells in shDSC1 3D-RHE was reduced. **I)** Distribution of detected division anlges is not changed in shDSC1 3D-RHEs after 6 days of cultivation. Ratio of symmetric (0°-30°), random (30°-60°) and asymmetic (60°-90°) cell division was not altered in shDSC1 rafts. Scale bar: 100 µm (black); 20 µm (white).

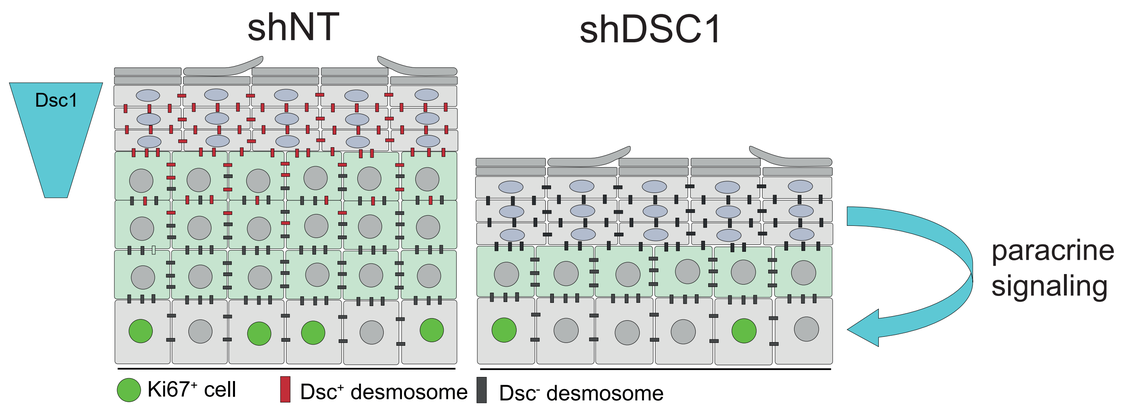
Differential gene expression in shDSC1 3D-RHEs



A) Volcano plot of differentially expessed genes in shDSC1 3D-RHE after 6 days of cultviation, confirming DSC1 downregulation in the RNAseq data. **B)** Gene set enrichment analysis (GSEA) comparing differentially expressed gene sets to published signatures of epidermal layer specific expression patterns from single cell RNAseq data (https://doi.org/10.1038/s41467-020-18075-7). shDSC1 shows significant downregulation of genes corresponding to basal layer subsets BASI and BASII as well as spinous layer SPNI (False discovery rate; FDR < 0.05). **C)** Comparison of up- and downregulated Hallmark gene sets in RNAseq analysis demonstrated alterations in pathways involving TGF-beta, TNF-alpha signalling and interferon responses, suggesting a paracrine regulation of keratinocyte proliferation dependent on the presence of DSC1.

Statistical analysis: All experiments were analyzed using a student`s t-test or two-way ANOVA (Sidak`s correction). Each data point represents single experiments, including two technical replicates per condition. Error bars represent the mean value ± SD, *p < 0.05.

Conclusion



Our data indicate that DSC1 modulates cell cohesion as well as proliferation and differentiation of keratinocytes in 3D-reconstructed human epidermis, which may be explained by a possible DSC1-dependent paracrine signaling.