Targeting phosphorylation of the keratin-desmosome complex for the treatment of Epidermolysis Bullosa Simplex

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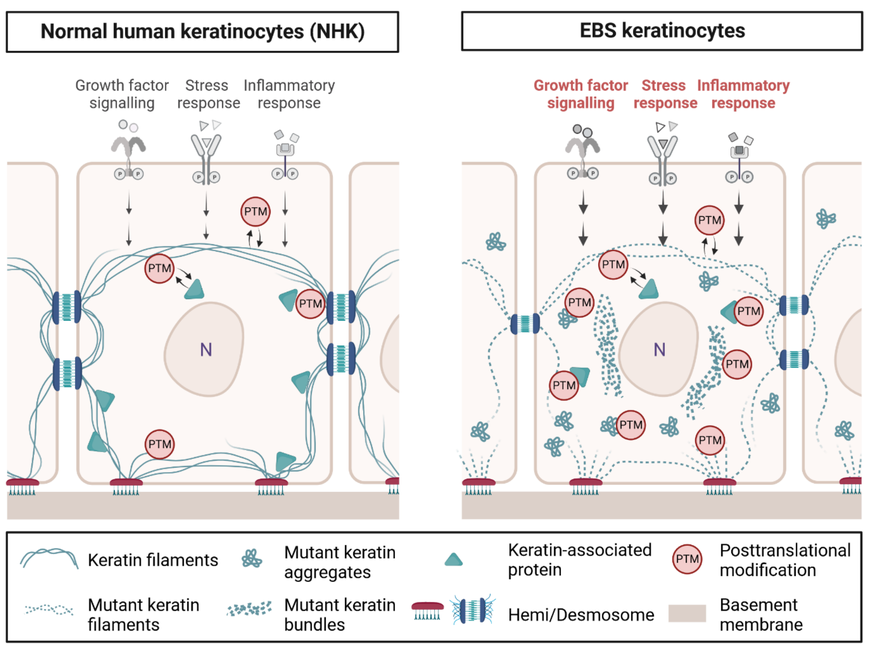
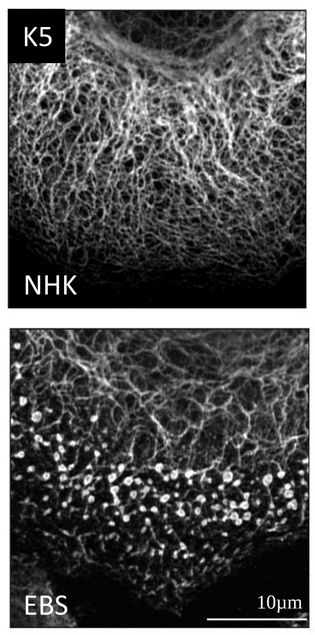
INTRODUCTION: Potential use of PTM targeting drugs as a future therapeutic approach for EBS



Epidermolysis bullosa simplex (EBS) is a rare, severe and potentially life-threatening disorder for which no adequate therapy exists. Most cases are caused by dominant mutations in **keratins *KRT5* or *KRT14***, characterized by cytoplasmic keratin aggregates, profound keratinocyte fragility and cytolysis. The keratin intermediate filament (KIF) organization is defined by: (i) its protein sequence, which includes sequence variants in EBS (dotted lines in model; *created with BioRender.com*) in comparison to normal human keratinocytes (NHK; straight lines), and (ii) the regulation by **posttranslational modification (PTM)**-adding and -removing enzymes and (iii) keratin-associated proteins. A precise regulation is critical for a functional keratin cytoskeleton, which is connected to hemi/desmosomes.

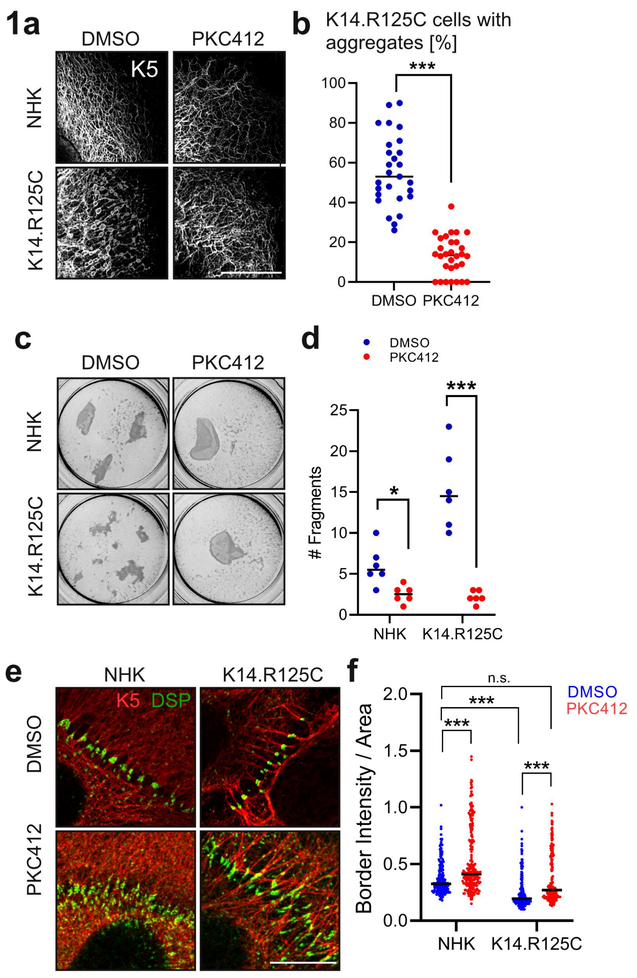
We and others have recently found that a subset of keratin mutations is accompanied by **elevated phosphorylation** of keratins and desmosomes in EBS-associated keratinocytes. We next showed that elevated phosphorylation of keratins and desmoplakin (DSP) reinforced the detrimental effect of keratin mutations on cell adhesion and tissue integrity, contributing to a more severe disease.

This led to our hypothesis that reducing phosphorylation of keratins and DSP by chemical compounds should improve tissue stability in EBS-associated keratinocytes.



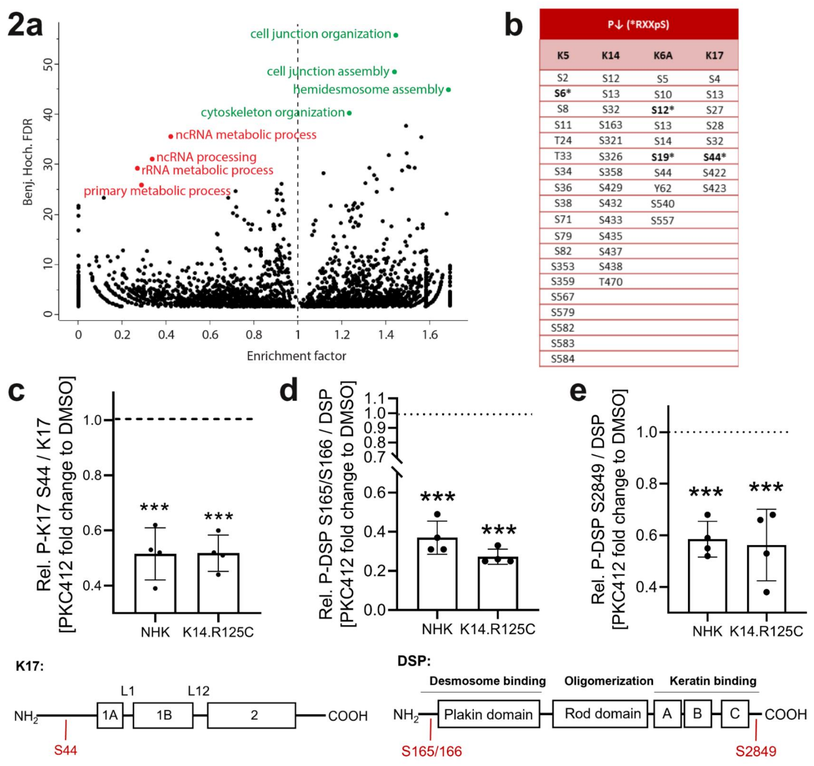
RESULTS: Targeting phosphorylation of the keratin-desmosome complex by the kinase inhibitor PKC412

1) Reduced keratin aggregates & improved intercellular cohesion:



(**1a,b**) The multi-kinase inhibitor PKC412 reduced keratin aggregation by 40% in patient-derived K14.R125C EBS-associated keratinocytes. (**1c,d**) Epithelial shear stress assays revealed that PKC412 restored intercellular adhesion. (**1e**) Confocal immunofluorescence shows PKC412- or DMSO-treated NHK and EBS cells stained with K5 and DSP. (**1f**) DSP fluorescence intensity along cell borders was quantified (>200 cell borders, *n*=3, 2way ANOVA, Sidak´s multiple comparisons test: ***P<0.001, n.s.=not significant).

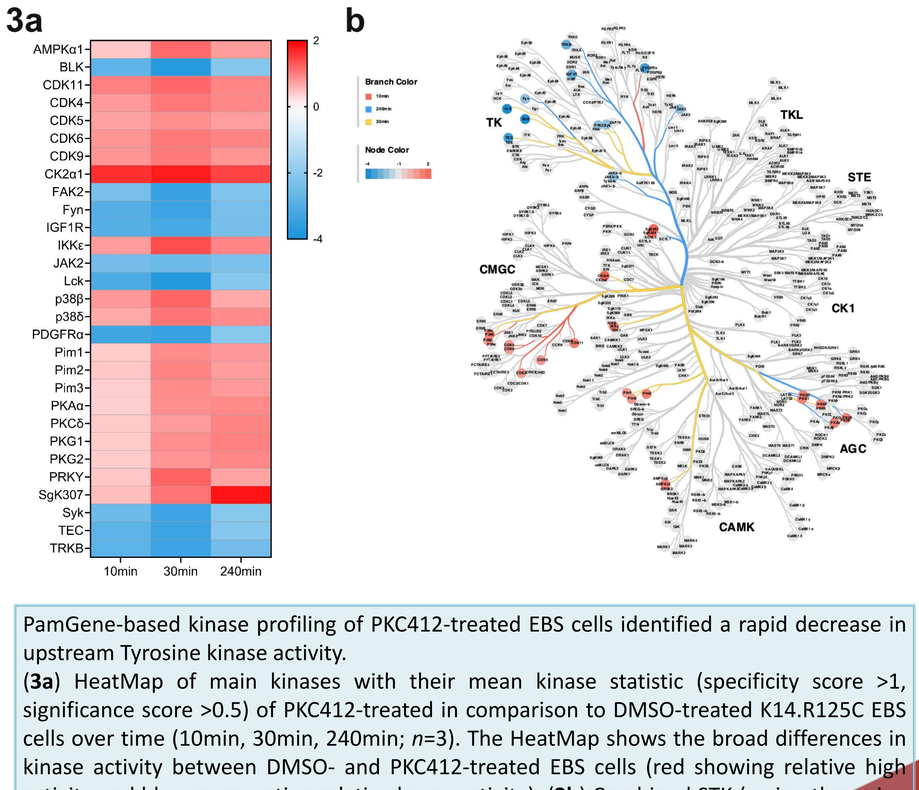
2) Altered keratin and desmoplakin (DSP) phosphorylation:



P↓ (*RXXpS)			
K5	K14	K6A	K17
S2	S12	S5	S4
S6*	S13	S10	S13
S8	S32	**S12***	S27
S11	S163	S13	S28
T24	S321	S14	S32
T33	S326	**S19***	**S44***
S34	S358	S44	S422
S36	S429	Y62	S423
S38	S432	S540	
S71	S433	S557	
S79	S435		
S82	S437		
S353	S438		
S359	T470		
S567			
S579			
S582			
S583			
S584			

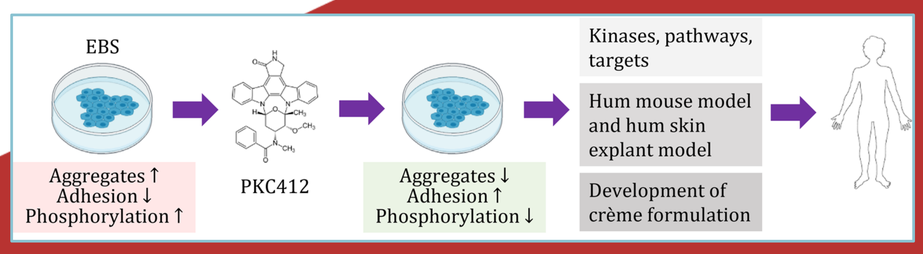
Global phosphoproteomic analysis together with immunoblots using phospho-epitope specific antibodies revealed that PKC412 treatment altered phospho-sites on keratins and DSP. (**2a**) GOBP annotation enrichment analysis. Fisher exact test using Benj. Hoch. FDR truncation value of 0.01. The most significantly regulated biological process categories are annotated in green (up) and red (down). (**2b**) Reduced serine (S), threonine (T) and tyrosine (Y) residues in K5, K14, K6A and K17, which were less phosphorylated upon PKC412 treatment in comparison to DMSO-treated EBS keratinocytes. (**2c-e**) Graphs depicting the indicated phospho-(P)-protein signal for K17 or DSP relative to total protein from PKC412-treated NHK and K14.R125C cells normalized to DMSO-treated cells (mean±SD, *n*=4, 2way-ANOVA, Sidak´s multiple comparisons test, ***P<0.001). **Below**: domain structures of K17 (left) and DSP (right) with indicated P-sites reduced upon PKC412 treatment (in red).

3) Altered kinase activities:



PamGene-based kinase profiling of PKC412-treated EBS cells identified a rapid decrease in upstream Tyrosine kinase activity.
(**3a**) HeatMap of main kinases with their mean kinase statistic (specificity score >1, significance score >0.5) of PKC412-treated in comparison to DMSO-treated K14.R125C EBS cells over time (10min, 30min, 240min; *n*=3). The HeatMap shows the broad differences in kinase activity between DMSO- and PKC412-treated EBS cells (red showing relative high activity and blue representing relative lower activity). (**3b**) Combined STK (serine threonine kinase) and PTK (protein tyrosine kinase) kinome tree. Top predicted kinases are represented on phylogenetic tree of the human protein kinase family. Node color denotes kinases statistic (PKC412- vs. DMSO-treated) and branch color time-dependency.

PERSPECTIVE: Towards a clinical study



We hypothesize that the severity of EBS and likely other keratinopathies results from an interplay of disease-associated mutations and consequent PTM alterations. Interfering with pathways that control keratin or keratin-associated protein PTMs provides a **novel opportunity for the development of molecular therapies** that offer local (e.g., application of an ointment or cream) or systemic (e.g., oral or intravenous administration) treatment of keratinopathies including EBS.

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Kinase Inhibition by PKC412 Prevents Epithelial Sheet Damage in Autosomal Dominant Epidermolysis Bullosa Simplex through Keratin and Cell Contact Stabilization. **Rietscher K**, Jahnke HG, Rübsam M, Lin EW, Has C, Omary MB, Niessen CM, Magin TM. J Invest Dermatol. 2022 Dec;142(12):3282-3293.

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