# Fibroblasts activation and abnormal extracellular matrix remodelling as common hallmarks in three cancer-prone genodermatoses

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# Introduction

## What's already known?

- Recessive dystrophic epidermolysis bullosa (RDEB), Kindler syndrome (KS) and Xeroderma pigmentosum C (XPC) are three genodermatoses with high predisposition to cancer development.

- Although their causal genetic mutations mainly affect epithelia, the dermal microenvironment likely contributes to the physiopathology of these disorders.

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# Objective

To investigate, by means of comparative gene expression analysis (RNAseq), the role played by dermal fibroblasts in the pathogenesis of RDEB, KS and XPC.

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# Methods

- ***Sample collection and cell culture***

Fibroblasts were isolated from skin biopsies (unaffected areas) of 7 healthy donors, 11 RDEB, 4 KS, and 4 XPC patients.

- ***RNA-Seq analysis***

-Total RNA was isolated from confluent primary fibroblasts.

-Examination of the differentially expressed genes, a functional enrichment analysis and a description of affected signaling circuits was carried out.

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# Methods

- ***Validation***

Transcriptomic data were validated at the protein level in cell cultures (Western blot), serum samples (ELISA) and skin biopsies (Immunofluorescence).

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# Results

- ***Identification of common expression signatures (I)***

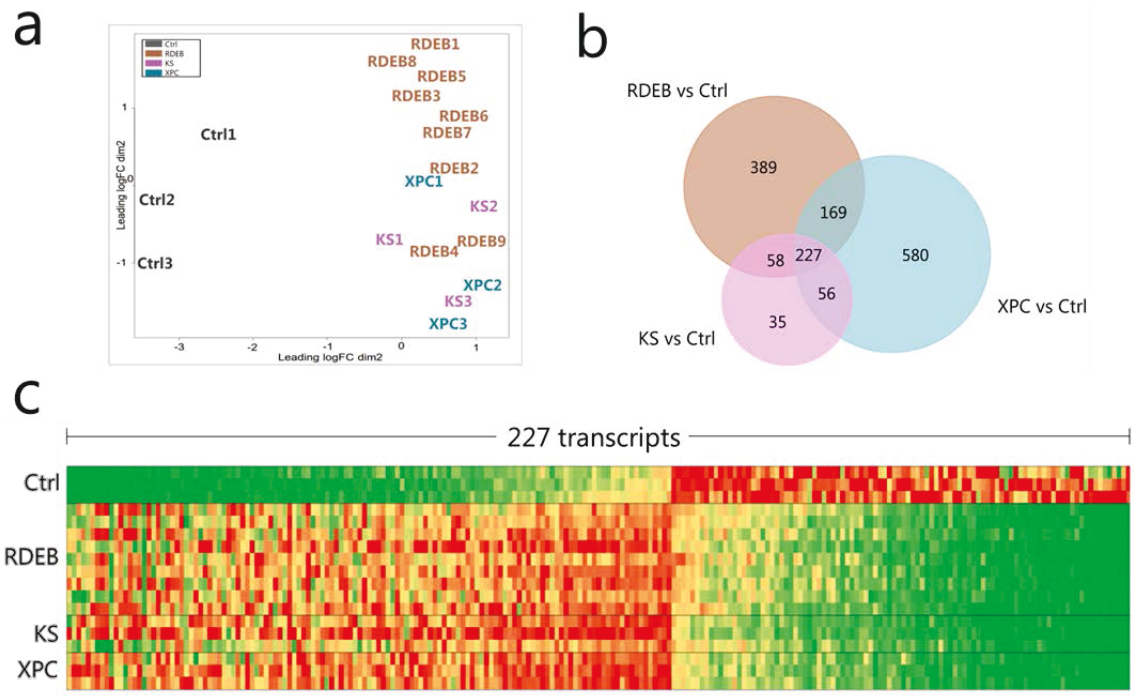


**Fig 1.** Differential gene expression profile. (a) Principal component analysis (PCA)-plot represents the global distribution of each sequenced sample. Disease samples tend to group together and distantly from the controls. (b) An overlapping set of 227 transcripts were commonly dysregulated in all the diseases (Venn diagram). (c) Heatmap of the normalized expression of the 227 transcripts (red: upregulated, green: downregulated in the disease).

- Transcriptomic data from the different genodermatoses are located close to each other within the plot, and separated from controls, pointing towards a similar transcription profile between them.

- Inter-disease comparisons against control fibroblasts revealed a common signature of 227 differentially expressed transcripts.

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# Results

- ***Identification of common expression signatures (II)***

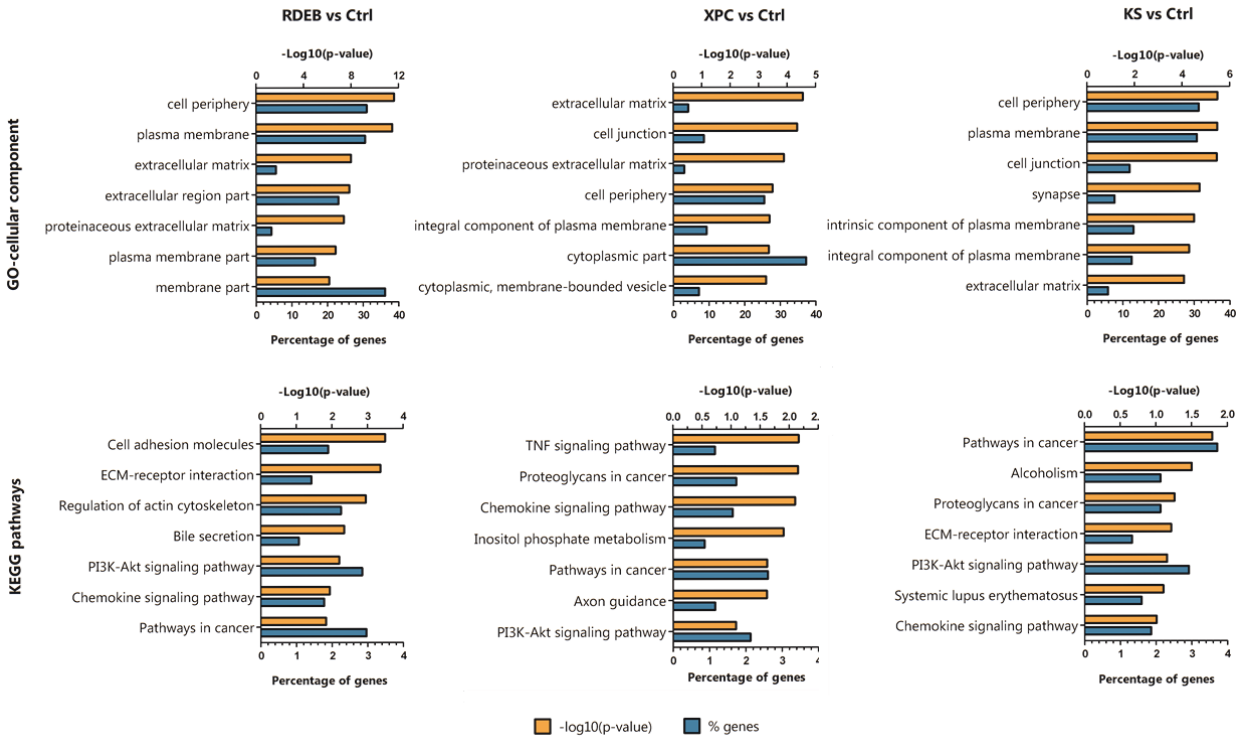


**Fig 2.** Enrichment analysis of differentially expressed genes (GO terms and KEGG pathways). The highest ranked categories in each disease are shown according to the p-value and percentage of genes.

- The dysregulated genes were significantly associated with extracellular matrix and cell periphery (enriched GO terms).

- KEGG pathway analysis identified common alterations in apoptosis (PI3K-Akt) signalling, chemokine repertoire and two cancer-related pathways.

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# Results

- ***Identification of common expression signatures (III)***

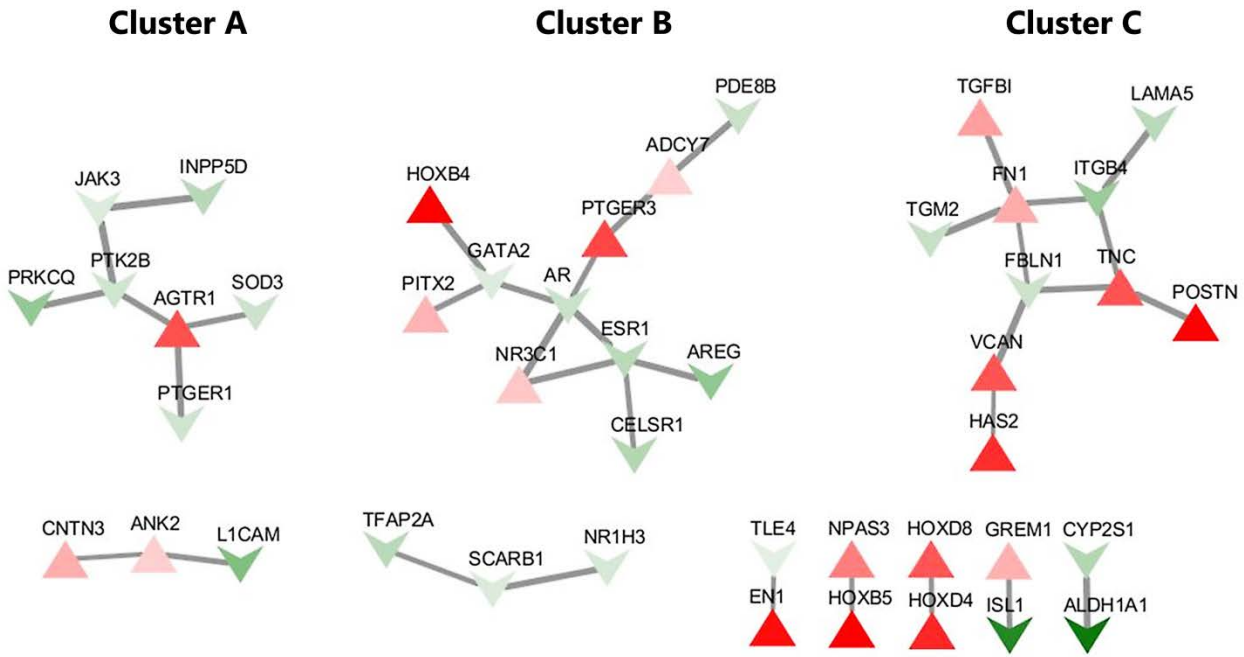


**Fig 3.** Protein-protein interaction network of the common dysregulated genes. The three largest connected components, labelled as “Cluster A, B and C”, represent a cluster of highly-connected, biologically related proteins. Node colour is graded according to the average fold change (red: upregulated, green: downregulated in the disease). Edge width is proportional to STRING interaction score, which represents the confidence for that interaction.

- Protein-protein interaction (PPI) networks were generated to identify functional associations between the differentially expressed genes.
- Cluster C is represented by genes implicated in the deposition and remodelling of the ECM.
- Among the minor-clustered nodes, downregulation of the antioxidant enzyme ALDH1A1 stands out (the most dowregulated gene).

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# Results

- ***Validation of altered gene expression profile (Cluster C from PPI data)***

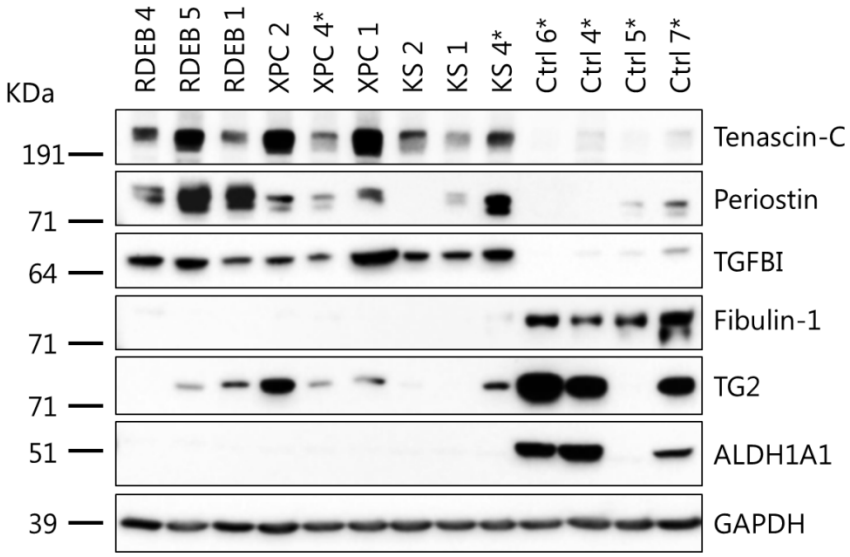


**Fig 4.** Western blot confirm the high expression of tenascin-C, periostin and TGFBI, together with an underexpression of TG2, ALDH1A1 and fibulin-1 in samples from RDEB, XPC and KS patients. (*) Additional samples not used in the RNA-Seq .

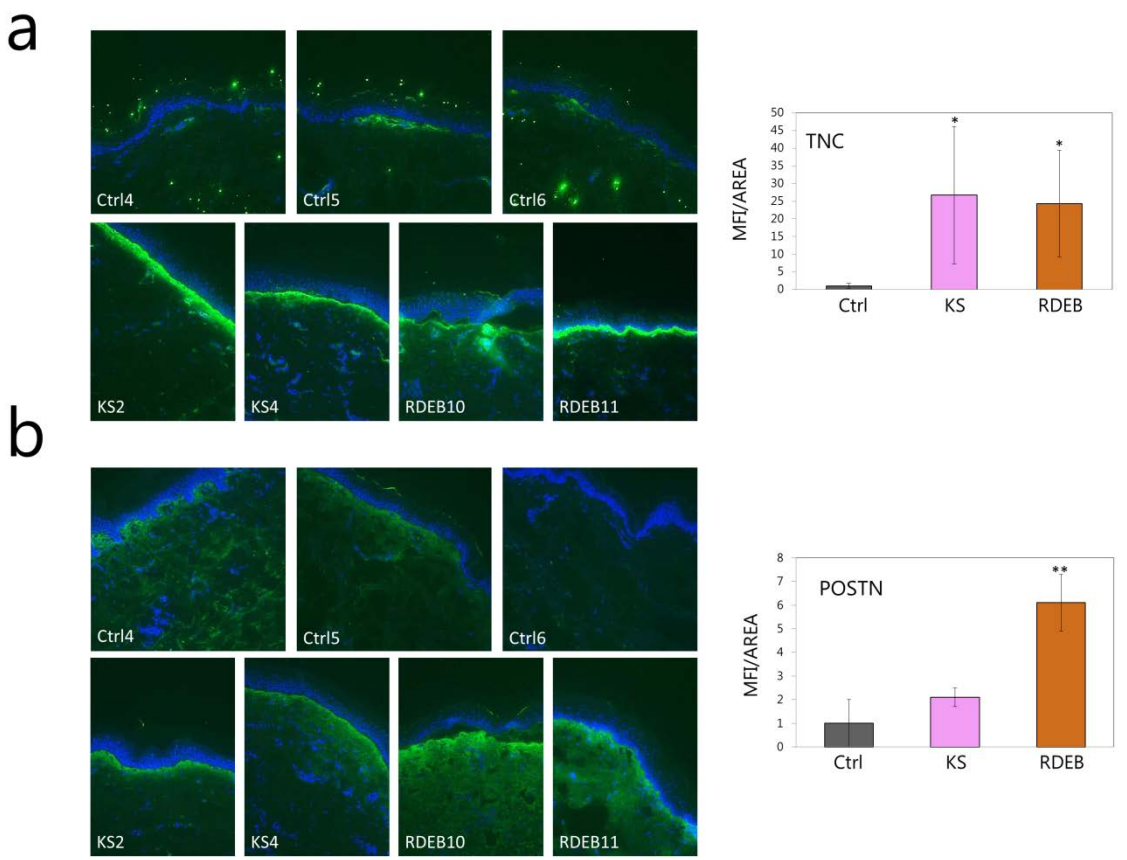


**Fig 5.** Skin biopsies sections from RDEB, KS and healthy controls were stained for (a) tenascin-C and (b) periostin. Quantitation of fluorescence intensity was measured and represented as mean staining intensity/area value +/- SD. *p<0.05; ** p<0.01.

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# Results

- ***Identification of serum periostin as a novel biomarker in RDEB***

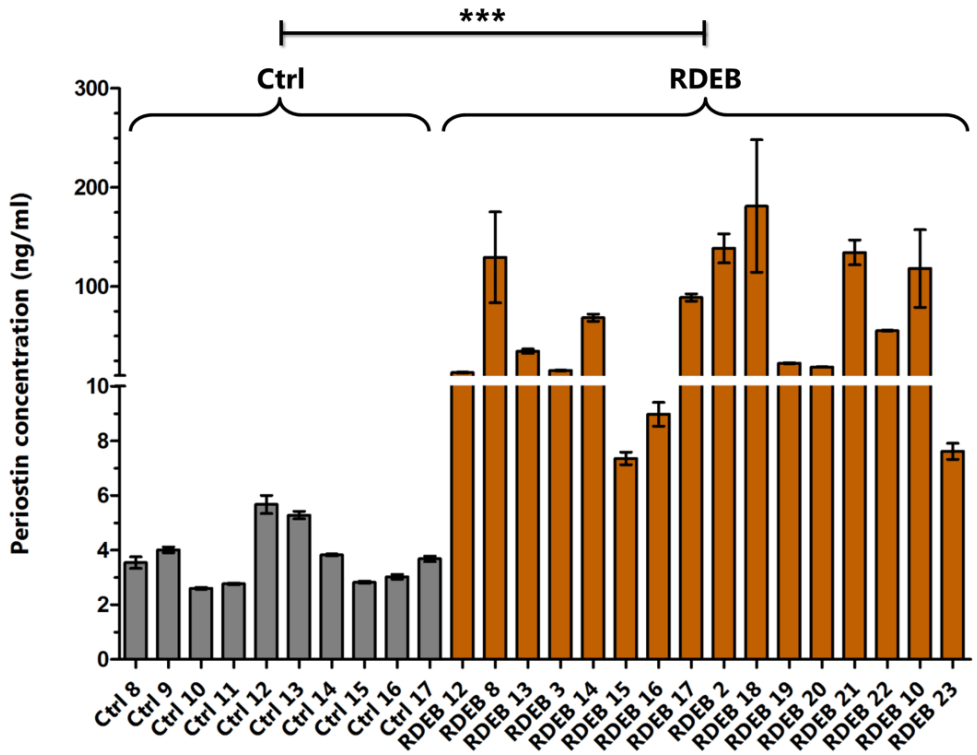


**Fig 6.** Circulating periostin levels in healthy volunteers and RDEB patients measured by an enzyme-linked immunosorbent assay (ELISA). ***p-value=0.0008

Circulating periostin concentration were remarkably higher in RDEB patients (65,69ng/ml ± 14.75 SEM) compared to controls (3.72ng/ml ± 0.33 SEM).

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# Discussion

- Bioinformatic analyses revealed the presence of three major determinants of an activated fibroblast phenotype, namely: increased cell survival, altered TGF-ß signalling, and abnormal extracellular matrix remodelling.

- Without excluding a triggering effect due to the primary deficiencies (i.e. loss of C7, XPC or kindlin-1), our results challenge the view of a direct genetic cause-driven effect.

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# Discussion

- Rather, they suggest the existence of a shared injury-responsive event able to transduce the primary defect into epigenetic changes leading to the acquisition of a synthetic fibroblast phenotype.

- All in all, the common genetic signature in fibroblasts of the three genodermatoses shares some similarities with those found in myofibroblasts, wound-activated fibroblasts and cancer-associated fibroblasts (CAFs).

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# Conclusions

## What does this study add?

- This study disclose a large gene transcription overlapping profile between XPC, KS and RDEB fibroblasts that points towards an activated phenotype with high matrix-synthetic capacity.

- This common signature seems to be independent of the primary causal deficiency, but reflects an underlying derangement of the extracellular matrix via TGF-β signalling activation and oxidative state imbalance.

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# Conclusions

## What is the translational message?

- This study broadens the current knowledge about the pathology of these diseases and highlights new targets and biomarkers for effective therapeutic intervention.

- It is suggested that high levels of circulating periostin could represent a potential biomarker in RDEB.

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