Molecular and mutational signatures of squamous cell carcinomas in epidermolysis bullosa

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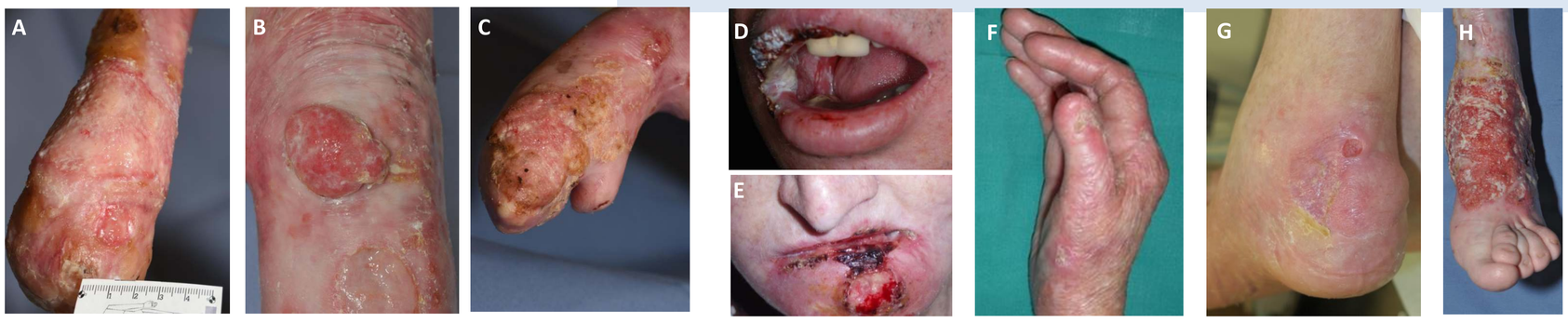
Background and aim of the study

- EB is a heterogeneous group of genetic skin disorders associated with widespread blisters due to fragility of the skin. There are four major EB types depending on the level of cleavage in the skin: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (KS)[1].
- Squamous cell carcinoma (SCC) is one of the most severe, life-limiting complication of EB, especially in recessive DEB (RDEB)[2]. Surgical excision with clear margins of SCC did not prevent the later occurrence of regional or distant spread from another hidden cutaneous SCC. Furthermore, once the patient develops metastatic disease, there are no interventions which proved to be effective[3]. Thus, there is a high unmet therapeutic need.
- We analyzed molecular and mutational signatures of 48 SCC obtained from patients with EB, 10 with RDEB, seven with KS and one patient with JEB in order to clarify molecular pathomechanisms and identify targets for therapy.

Statements

- All EB SCC samples showed high EGFR and COX-2 expression, and expression of at least one immune checkpoint, CTLA-4, PD-1 or PD-L1.
- The expression of IDO, CTLA-4 and PD-L1 were significantly higher in RDEB SCC compared to KS SCC.
- The molecular biomarker expression was higher in tumor samples compared to non tumor samples in RDEB patients.
- Mutational signatures of EB SCC were similar to those of head and neck SCC and UV SCC. KS SCC showed higher tumour mutational burden as compared to RDEB SCC and JEB SCC.
- Recurrently altered genes (*TP53*, *CDKN2A*, *NOTCH1/2*, *KNSTRN*) but also a wide spectrum of oncogenic mutations were identified, affecting cell cycle, DNA damage response, tyrosine kinases, PI3K-AKT-mTOR and RAF-MEK-ERK pathways in all EB SCC.

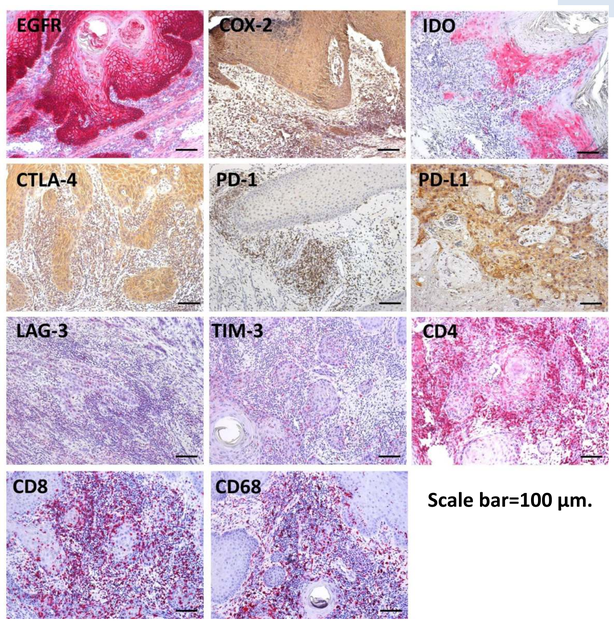
Clinical and pathological features of the EB SCC cohort



The most common sites of EB SCC are limbs. EB SCCs are mostly well-differentiated, but very aggressive, with poor prognosis.

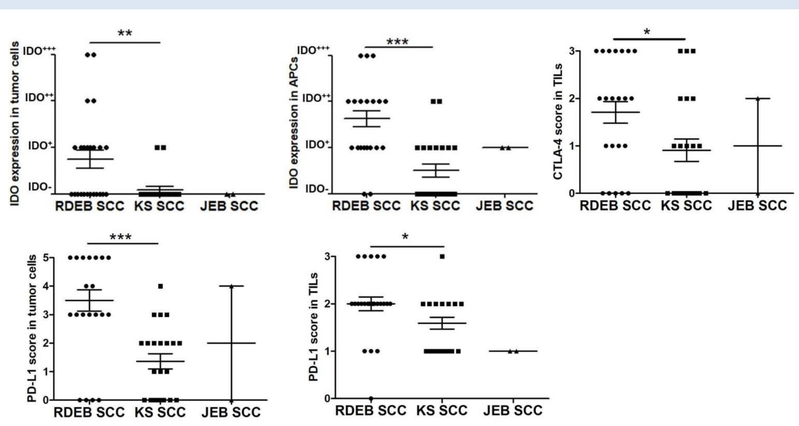
Figure 1. EB SCC clinical manifestations. A: SCC on scarring and chronic wounds on extremities in RBEB SCC; B: tumoral RDEB SCC; C: hyperkeratotic lesion of RDEB SCC; D: a KS SCC localized in the oral cavity; E: a KS SCC localized on the lip; F: a KS SCC localized on the right hand; G: a KS SCC localized on the right foot. H: a JEB SCC localized on the left foot.

Expression of molecular biomarkers in EB SCC



Scale bar=100 µm.

Figure 2 (left panel). Examples of IHC staining of EBS SCC. All RDEB SCC stained positive for EGFR and for at least one immune checkpoints. The inflammatory infiltrate was CD4, CD8 and CH68 positive and CD20 negative. LAG-3 and TIM-3 showed variable expression. CD39 (ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1) which converts ATP into AMP was negative.



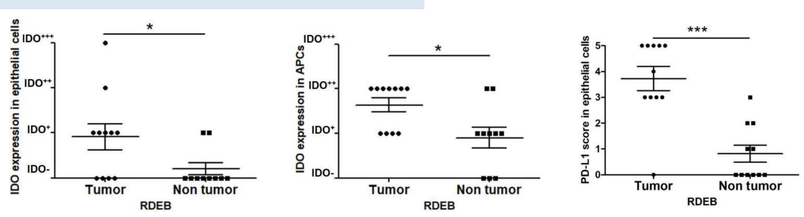


Figure 3 (middle panel). The expression of immune biomarkers with significant differences in different EB SCC. The IDO expression in tumor cells and APCs, PD-L1 expression in tumor cells and TILs, and CTLA-4 expression in TILs were significantly higher in RDEB SCC than in KS SCC. **P*<0.05, ***P*<0.01, ****P*<0.001

Figure 4 (right panel). The expression of immune biomarkers with significant differences between tumor and non tumor samples in RDEB patients. The non tumor tissue samples from RDEB patients also showed molecular biomarker expression but at a low level. IDO expression, and PD-L1 expression in epithelial cells were significantly higher in tumor samples than those in non tumor tissue samples. Expression of other molecular biomarkers was higher in tumor samples than in non tumor tissue samples. **P*<0.05, ***P*<0.01, ****P*<0.001.

Tumor mutational burdens and mutational signatures in EB SCC

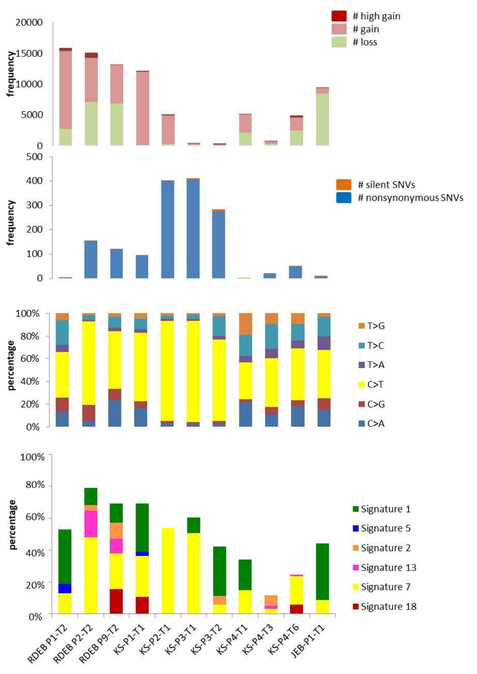


Figure 5. Overview of somatic variants in EB SCC. The top two stacked histograms show the number of each type of SNV and CNV per sample. The bottom two plots show the percentages of nucleic acid exchanges and mutational signatures across the samples.

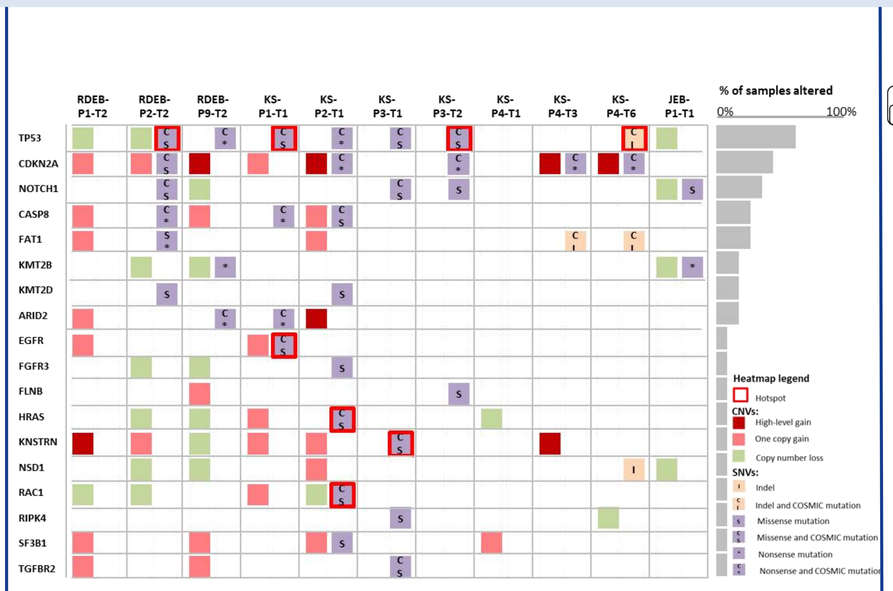


Figure 6. Integrated view of recurrently altered genes in EB SCC.

Heatmap representation of selected significantly altered genes in SCC. CNVs are colored in red for high-level amplification events. Light red and light green to represent low-level CNVs. SNVs are colored by type in purple and orange, and also labeled: I for insertion or deletion (indel), S for missense, C for COSMIC, * for nonsense, and red frame for hotspot. The genes are listed in the order of decreasing number of alterations across the samples, as shown on the right-hand plot. Note that each heatmap is sorted independently across the samples, to best illustrate the pattern of mutations, such as mutual exclusivity or concurrence.

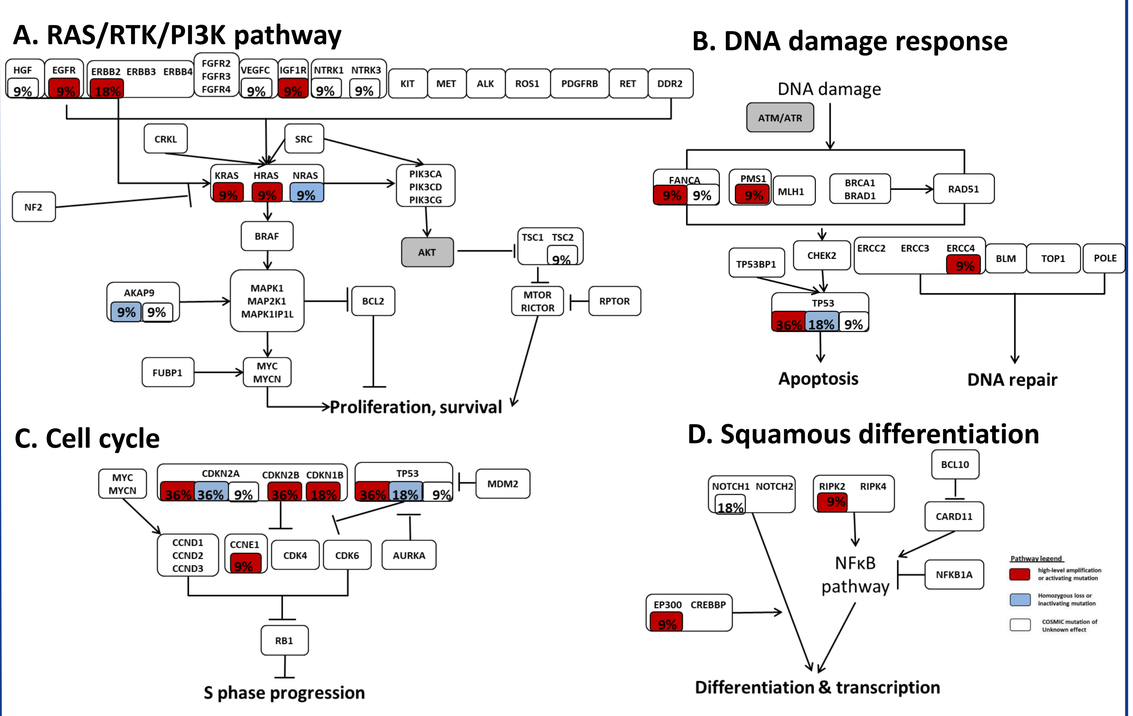


Figure 7. Altered pathways in EB SCC.

Pathway diagrams depicting the percentage of samples with alterations in A) RAS/RAF/MEK/ERK and PI3K/AKT signaling; B) DNA damage response; C) cell cycle, and D) squamous cell differentiation. Alterations are classified as activating (high-level amplification or known activating mutation colored red), inactivating (homozygous loss or nonsense mutation colored blue), or potentially cancer associated (COSMIC mutation colored white).

References

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