Combined loss of Trex2 and DNase1L2 nucleases leads to disrupted DNA degradation during lingual cornification and absence of inflammatory responses

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Abstract

Nuclear destruction is one of the main changes occurring throughout keratinocyte cornification, a differentiating-associated mode of programmed cell death. However, the mechanisms of DNA degradation during cornification remain unclear. Here, we investigated the effects in mice of simultaneous loss of the two keratinocyte-specific nucleases, the DNase1L2 endonuclease and the Trex2 exonuclease. Studies in single and double deficient mice have revealed that Trex2 and DNase1L2 contributed and cooperated in the DNA degradation process taking place during keratinocyte cornification in the tongue epithelium, whereas were dispensable for homeostatic epidermal skin cornification. Notably, simultaneous loss of Trex2 and DNase1L2 led to synergistic accumulation of DNA fragments into the uppermost lingual epithelium layers, rather than a simple sum of single DNase1L2 and Trex2 loss phenotypes, pointing out these two nucleases as essential members of the DNA degrading machinery in lingual cornification process. Intriguingly, the abnormal accumulation of cytosolic DNA in lingual keratinocytes was not associated to DNA-driven immune responses and inflammation, establishing that lingual epithelium displays physiological tolerance to aberrantly retained endogenous DNA. Keratinocyte immunological tolerance to self-DNA may be explained in part by the low expression levels of key DNA sensing and signalling genes. In sum, our data indicate a keratinocyte tissue-dependent essential interplay of Trex2 and DNase1L2 in DNA degradation during homeostatic lingual cornification.

1 Trex2 exonuclease and DNase1L2 endonuclease are expressed mostly in the differentiating keratinocytes of the skin and tongue epithelia

DNase1L2 and Trex2 immunofluorescence images showing nucleases (red) and DNA (blue) staining in the back skin and tongue sections from wild-type (wt), *Dnase1l2*^{-/-} and *Trex2*^{-/-} mice, as indicated. Scale bars = 25 μm. Dashed lines, epidermal-dermal border; continuous lines, bottom of the stratum corneum; dotted lines, upper border of the stratum corneum.

2 Double Trex2 and Dnase1l2 loss leads to an increase of fragmented DNA in the upper keratinocyte layers of the tongue, but not of the epidermis

TUNEL labelling of free 3'-OH DNA ends (red) and nuclei (blue) counterstained with Hoechst of back and ear skin, and the filiform papillae and proximo dorsal side tongue sections from wt, *Trex2*^{-/-}, *Dnase1l2*^{-/-} and *Trex2*^{-/-}*Dnase1l2*^{-/-} mice. Back and ear skin samples were from 6 days-old mice. Tongue samples were from 7-9 weeks-old mice. Scale bars = 25 μm. Dashed lines, epidermal-dermal border; continuous lines, bottom of the stratum corneum; dotted lines, upper border of the stratum corneum.

3 DNA-driven gene expression signature is not induced in the tongue of the *Trex2*^{-/-}*Dnase1l2*^{-/-} mice

Expression of the indicated genes in the tongue from wt, *Trex2*^{-/-}, *Dnase1l2*^{-/-} and *Trex2*^{-/-}*Dnase1l2*^{-/-} mice, as determined by RT-qPCR. Each dot indicates a sample from an individual mouse, and horizontal lines represent the mean value. Significant differences, by the Mann-Whitney test between genotypes: *, $P < 0.05$; ***, $P < 0.001$.

4 Intracellular DNA does not induce a transcriptional type I IFN response in mouse keratinocytes

Mouse bone marrow-derived macrophages (BMDM) and keratinocytes cells were untreated or treated with Lipofectamine® LTX (2 ml/ml), genomic DNA (2 mg/ml), premixed with Lipofectamine® LTX, LPS (10 ng/ml), TNFα (50 ng/ml) or a combination of these agents as indicated for 6 h prior to lysis. Total RNA was isolated, and mRNA levels of the indicated genes were quantified by RT-qPCR. Graphs show mean and SEM of at least three samples for each condition.

5 Model for the interplay between DNase1L2 and Trex2 in the DNA degradation during cornification of lingual keratinocytes

DNA is first fragmented by the endonucleolytic activity of DNase1L2 and subsequently degraded by the 3'prime exonuclease Trex2. At least one more DNA fragmentation process (not depicted here) is active to generate 3'-OH DNA fragments that cannot be further degraded *Trex2*^{-/-}*Dnase1l2*^{-/-} mice.

Conclusions

DNase1L2 and Trex2 **cooperate** in the breakdown and degradation of DNA during terminal differentiation of lingual keratinocytes.

Aberrant DNA retention in the murine oral epithelium does not lead to inflammatory responses.

References

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