iRHOM2 regulation of loricrin in the epidermal barrier

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Introduction

- Tylosis with oesophageal cancer (TOC) is an autosomal-dominant syndrome comprising both cutaneous and oesophageal features, with an increased lifetime risk of oesophageal squamous cell carcinoma development
- Missense mutations in *RHBDF2*, the gene encoding iRHOM2, underlie TOC
- Changes observed in TOC palm and oesophageal epithelium are indicative of an altered epithelial barrier. Here, we investigate the observation of nuclear loricrin in TOC epidermis and oesophageal epithelium

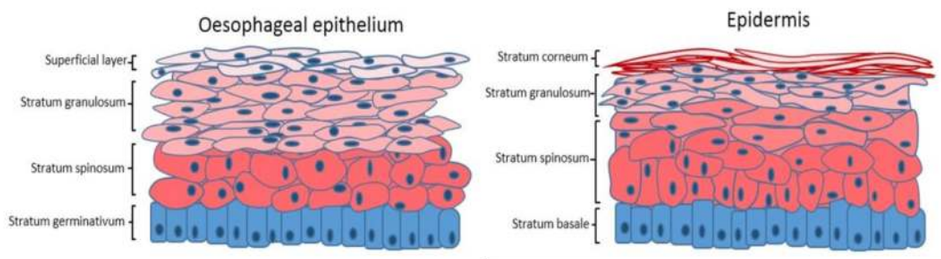


Fig 1: A schematic representation of the structural similarities between the oesophageal epithelium and epidermis

Results

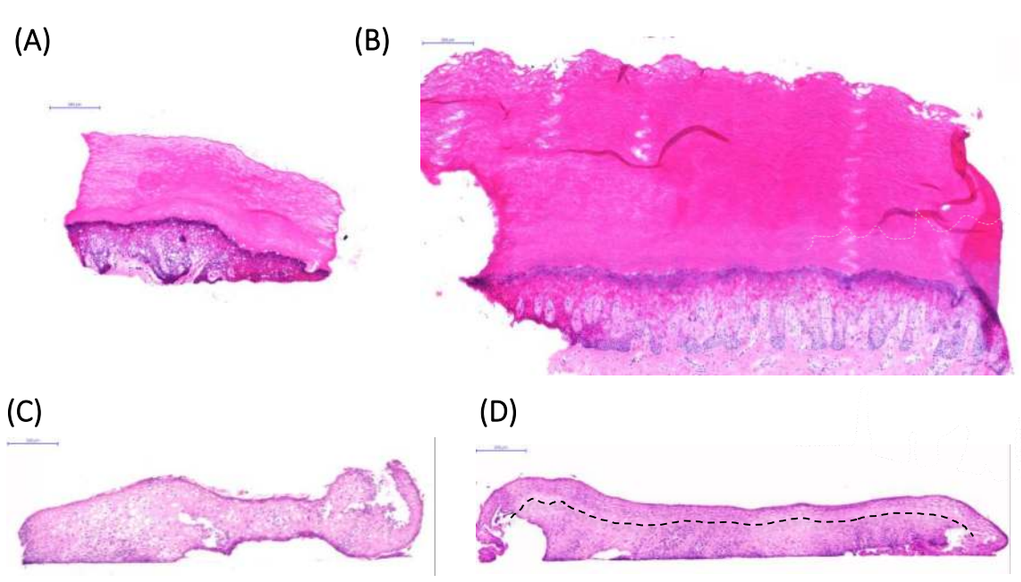


Fig 2: TOC palm and oesophagus exhibit thicker superficial layers when compared to control material

(A) H+E stained section of control palm epidermis
(B) H+E stained section of TOC epidermis showing considerable thickening of the Stratum corneum
(C) H+E stained section of control oesophageal epithelium
(D) H+E stained section of TOC oesophageal epithelium showing considerate thickening of the superficial cellular layer

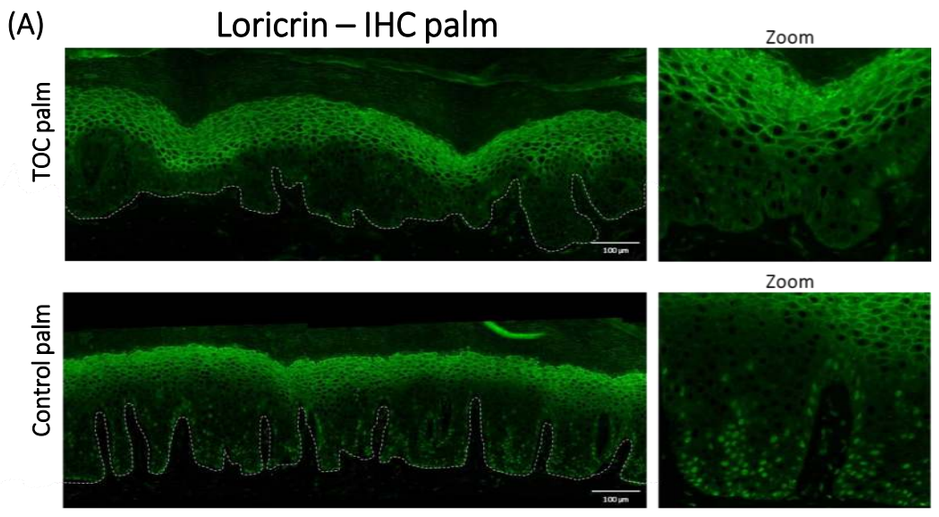
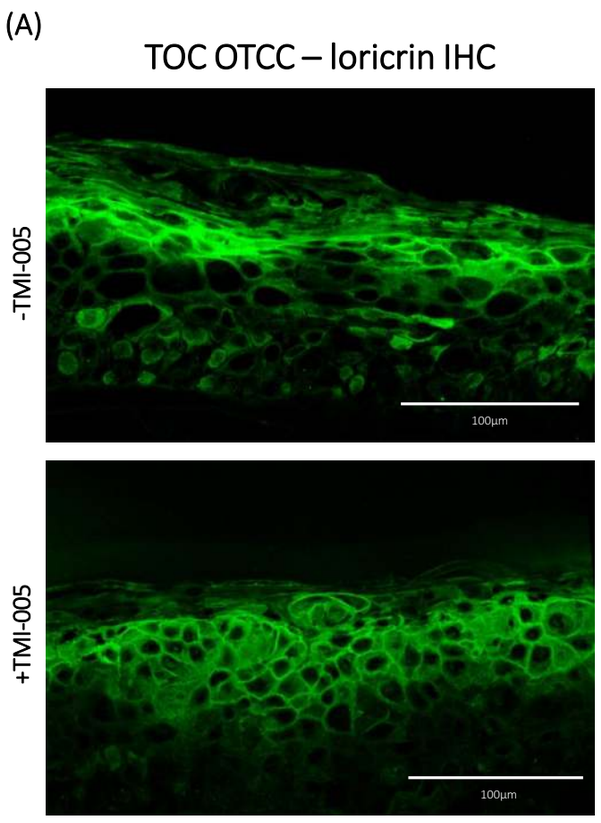


Fig 3: Immunohistochemical (IHC) staining of Loricrin shows a nuclear localisation in the TOC palm with TOC keratinocytes expressing high levels compared to controls

(A) TOC palm epidermis displays an increased level of nuclear loricrin at the basal layer when compared to control palm
(B) When cultured in monolayer, TOC keratinocytes express higher levels of loricrin
(C) The analysis of loricrin protein expression via western blotting shows under normal growth conditions, TOC cells express higher amounts of loricrin than the K17 cell line. The same trend is observed when cells are treated with PMA and TMI-005



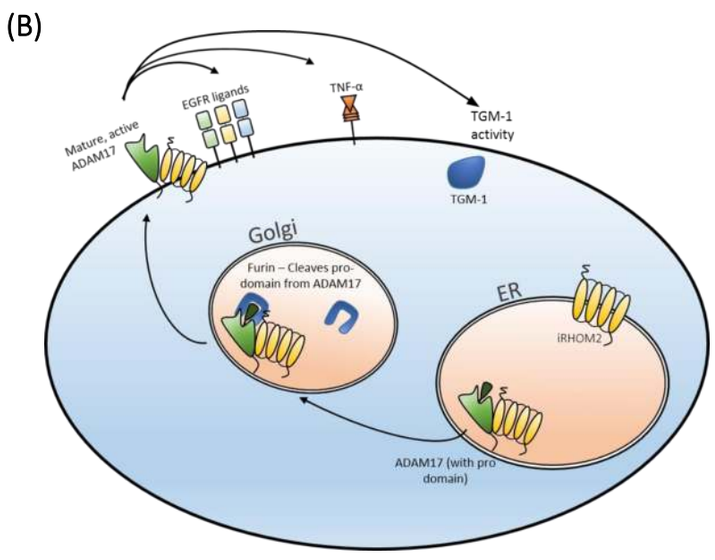


Fig 5: The cutaneous co-culture (OTCC) model mimics basal nuclear loricrin expression observed in TOC palm

(A) TOC OTCC's exhibit basal nuclear loricrin staining. When treated with TMI-005, loricrin is expressed strongly at the cell membrane and cytoplasm
(B) Schematic representation of the iRHOM2/ADAM17 pathway that is dysregulated in TOC patients due to a missense mutation in *RHBDF2*, the gene encoding iRHOM2. ADAM17 inhibition reduces cleavage of EGFR ligands and cytokine activation

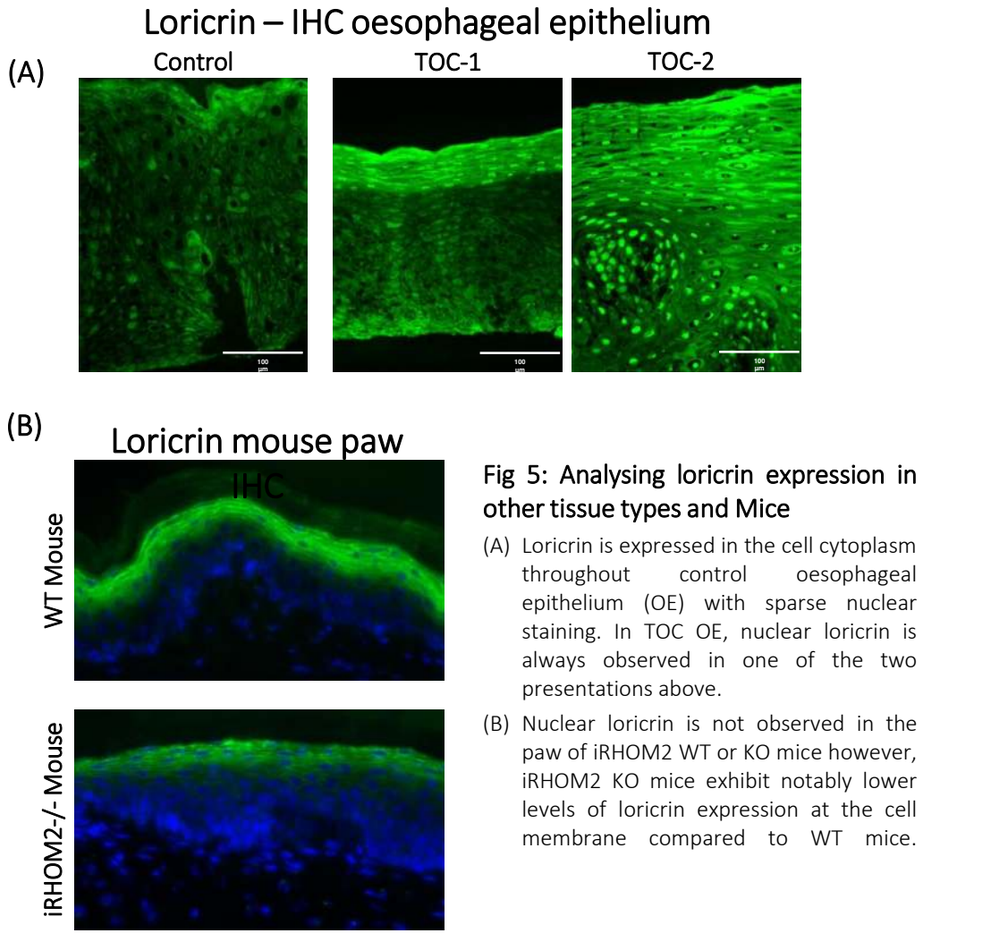


Fig 5: Analysing loricrin expression in other tissue types and Mice

(A) Loricrin is expressed in the cell cytoplasm throughout control oesophageal epithelium (OE) with sparse nuclear staining. In TOC OE, nuclear loricrin is always observed in one of the two presentations above.
(B) Nuclear loricrin is not observed in the paw of iRHOM2 WT or KO mice however, iRHOM2 KO mice exhibit notably lower levels of loricrin expression at the cell membrane compared to WT mice.

Conclusion

- Increased nuclear loricrin is observed in both TOC palm and oesophageal epithelium
- Nuclear loricrin is not observed in non lesional Interfollicular TOC or control skin, and is not observed in other PPK's we study
- Our cutaneous OTCC model is able to mimic the nuclear loricrin observed in TOC palm, the addition of TMI-005 eliminates this
- Mouse paw epidermis derived from an iR2 -/- mouse does not exhibit nuclear loricrin but shows loricrin is expressed at a much lower level compared to controls
- The mechanism by which loricrin locates to the nucleus in TOC material remains unknown, but highlights a previously unknown interaction between the iRHOM2/ADAM17 cell signalling pathway and loricrin

Acknowledgements

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