Identifying Cell Adhesion Defects In Keratinocytes Expressing TP63 Mutations Linked to Ectodermal Dysplasias

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ABSTRACT
Ankyloblepharon-ectodermal defects-cleft lip/palate (AEC) and Ectodactyly-ectodermal clefting syndrome (EEC) are ectodermal dysplasias characterized by a series of developmental abnormalities involving the skin, sweat glands, and nails, as well as other ectodermally-derived tissues. They are caused by mutations in the transcription factor TP63, which has been shown to be a master-regulator of epidermal development. One of the most severe clinical symptoms is the presence of skin erosions caused by severe skin fragility. This can lead to multiple clinical issues including lack of skin barrier function, skin blistering, and painful wounds. Our previous research has demonstrated abnormalities in the structure and function of desmosomes in AEC patients. We propose that cell adhesion defects also occur in EEC patients. To test this hypothesis, we used lentiviral constructs expressing AEC and EEC TP63 mutations in keratinocytes in order to study the effects within the context of the cell adhesion system. We will determine the expression and localization of adhesion proteins in these cells using western blotting and immunofluorescence techniques. We also expect to observe cell adhesion defects in the EEC mutations, which will be tested using a dispa functional assay. This research will further our understanding of the pathological process underlying ectodermal dysplasias, which is an important first step to design new treatment options for these devastating diseases.

HYPOTHESIS
We propose that skin fragility in EEC patients is caused by cell adhesion defects, which could include cell to cell and cell to the extracellular matrix adhesion.

METHODOLOGY & APPROACH

Step 1: Cells from a cultured keratinocyte cell line, known as NTERTs, were infected with a lentivirus that uses a K14 promoter (specific to keratinocytes) to drive expression of TP63 and TdTomato. Five virus constructs were utilized, with the only difference among them being the TP63 mutation. The virus construct is depicted below:

Step 1b: If needed, a FACS sorting was conducted using the Tomato fluorescence expression demonstrated above. TdTomato expression indicates cells that were transduced with the virus. Using immunofluorescence imaging, we analyzed Tomato and TP63 expression and localization after the virus had infected our NTERTs.

RESULTS
For our experiment, we analyzed the effects of TP63 mutations on NTERT cells. The NTERT keratinocyte cell line displays characteristics of a primary keratinocyte.

For our immunofluorescent staining procedure, we assessed proteins localized in the desmosomes.

REFERENCES

