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

Shirley Parraga[1], Saiphone Webb[2], Christina Sheldon[2], Maranke Koster[2], Peter Koch[2]

[1] Research Scholar Program Brody School of Medicine

[2] Department of Anatomy and Cell Biology Brody School of Medicine

ABSTRACT

Ankyloblepharon-ectodermal defects-cleft lip/palate (AEC) and Ectrodactyly-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 dispase 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.

INTRODUCTION

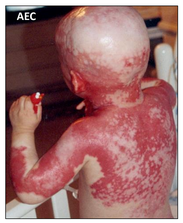
Clinical Presentation:

• Ankyloblepharon Ectodermal Defects-Cleft Lip/Palate Dysplasia (AEC) and Ectrodactyly Ectodermal Clefting Dysplasia (EEC) patients present with clinical manifestations including severe skin lesions, abnormal development of ectodermal appendages, and increased risk of skin infections.

• These symptoms occur within a spectrum of clinical manifestations with overlap in symptoms between AEC and EEC patients.

• AEC patients characteristically display skin erosions.

• EEC patients display ectrodactyly with more severe eye and limb abnormalities, but some EEC patients can also display skin erosions through unknown causes



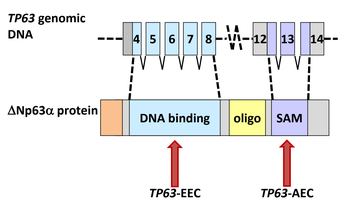
Role of TP63:

Referred to as the “master regulator” of epidermal development

Mutations in this protein result in varying effects on cell adhesion structures and functions

The pathways of TP63 responsible for disease progression are not fully understood.

The location of AEC and EEC mutations are indicated below:



HYPOTHESIS

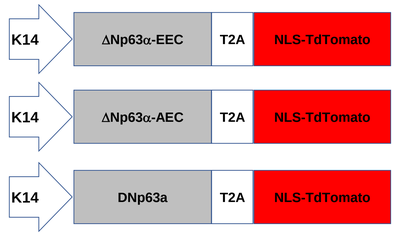
We propose that skin fragility in EEC patients is caused by cell adhesion defects, which could include cell to cell and cell to the extracellular matrix adhesion.

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

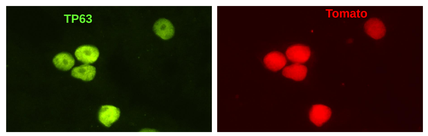


*** Our experiment design conducted keratinocyte infections using 2 constructs with AEC mutations, 2 constructs with EEC mutations, and a WT construct.**

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.



*** Image provided from a previous experiment conducted on 293 T cells using a different promoter, but with the same virus construct used in our experiment.**

Step 2:

Through immunofluorescent staining, we were able to track desmosomal (Desmoglein 1/2, Desmoglein 3, Desmocolin 3, Desmoplakin, and Plakoglobin) and keratin (Keratin 1 #87 and Keratin 14) proteins.

Step 3:

Conduct a western blot using cells transduced with the AEC, EEC, and WT lentiviral constructs to interpret protein expression for AEC and EEC mutations.

Step 4:

Conduct a dispase functional assay to determine whether cell adhesion defects exist in both AEC and EEC mutations.

RESULTS

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

Immunofluorescent image demonstrating the control conducted under low calcium conditions to ensure NTERT cells have keratin 14 and P63 expression in tact.

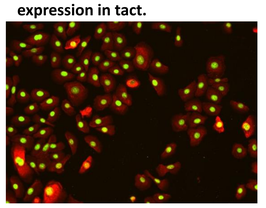
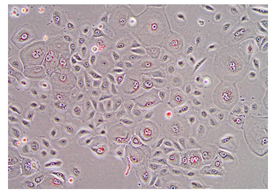
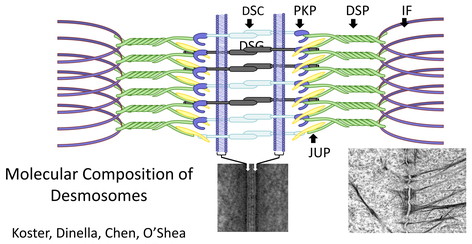


Image of cells demonstrating Tomato expression, indicating that the cells are transduced with our lentiviruses.



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

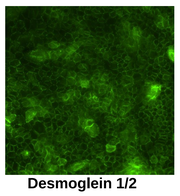


Molecular Composition of Desmosomes

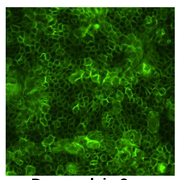
Koster, Dinella, Chen, O’Shea and Koch, 2014 (modified)

Schematic of desmosomal protein arrangement including Descolin (DSC), Desmoglein (DSG), Plakoglobin (PKP), Desmoplakin (DSP), Junctional Plakoglobin (JUP), and Intermediate Filaments (IF).

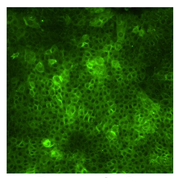
Staining Control Results:



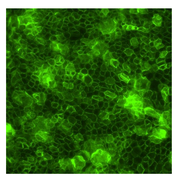
Desmoglein 1/2



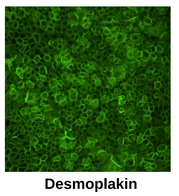
Desmoglein 3



Desmocolin 3



Plakoglobin



Desmoplakin

The images included in this section are examples of the stainings conducted so far. Once the cells infected with the virus described in the Methodology and Approach section are properly cell sorted, we will repeat the staining protocols with those cells.

We expect these defects in the AEC constructs, and we will test our hypothesis that skin fragility induced by EEC mutations might also be due to desmosomal defects, however, there may other potential causes we can consider which involve other cell adhesion systems.

PROJECT GOALS

• To validate five lentiviral constructs expressing AEC, EEC, and WT versions of TP63.

• To test whether all viral constructs resulted in infection as demonstrated by the expression of Tomato-positive cells.

• To compare expression and localization of cell adhesion and keratin markers between AEC and EEC mutation-expressing cells through immunofluorescence staining techniques.

• To observe the effects of TP63 mutations on cell lines induced into high calcium differentiation conditions for the purposes of analyzing and comparing expression of desmosomes and other cell adhesion markers.

• To interpret desmosomal protein expression levels for AEC and EEC mutations through western blotting techniques.

• To assess whether cell adhesion is functionally impaired in AEC and EEC mutations by using a dispase assay.

• To use our findings to further our understanding of the pathological process of TP63 mutations leading to AEC and EEC ectodermal dysplasias.

REFERENCES

Dinella JD et al. 2018. A human stem cell-based system to study the role of TP63 mutations in ectodermal dysplasias. Journal of Investigative Dermatology 138(7):1662-1665.

Koch PJ, Dinella J, Fete M, Siegfried EC, Koster MI. 2014. Modeling AEC—New approaches to study rare genetic disorders. Am J Med Genet Part A 164A:2443–2454.

Koster MI et al. 2014. Integrating animal models and in vitro tissue models to elucidate the role of desmosomal proteins in diseases. Cell Communication and Adhesion. 21(1):53-63.