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Modeling AEC—New Approaches to Study Rare Genetic Disorders

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Abstract

Ankyloblepharon-ectodermal defects-cleft lip/palate (AEC) syndrome is a rare monogenetic disorder that is characterized by severe abnormalities in ectoderm-derived tissues, such as skin and its appendages. A major cause of morbidity among affected infants is severe and chronic skin erosions. Currently, supportive care is the only available treatment option for AEC patients. Mutations in *TP63*, a gene that encodes key regulators of epidermal development, are the genetic cause of AEC. However, it is currently not clear how mutations in *TP63* lead to the various defects seen in the patients' skin. In this review, we will discuss current knowledge of the AEC disease mechanism obtained by studying patient tissue and genetically engineered mouse models designed to mimic aspects of the disorder. We will then focus on new approaches to model AEC, including the use of patient cells and stem cell technology to replicate the disease in a human tissue culture model. The latter approach will advance our understanding of the disease and will allow for the development of new in vitro systems to identify drugs for the treatment of skin erosions in AEC patients. Further, the use of stem cell technology, in particular induced pluripotent stem cells (iPSC), will enable researchers to develop new therapeutic approaches to treat the disease using the patient's own cells (autologous keratinocyte transplantation) after correction of the disease-causing mutations.

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Keywords

ectodermal dysplasia; ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC); TP63; induced pluripotent stem cells; iPS cells; iPSC-derived keratinocytes; iPSC-based cell therapy; skin equivalents; genodermatoses; inherited skin disorders; in vitro disease models

INTRODUCTION

Ectodermal dysplasias (ED) represent a group of inherited disorders that are characterized by developmental abnormalities in ectoderm-derived tissues and organs, including the epidermis and ectodermal appendages, such as hair follicles. Approximately 200 distinct ED have been described, however, the underlying genetic mutations have been identified only in approximately 30% of these disorders [Priolo, 2009; Visinoni et al., 2009]. Mutations in the *TP63* gene have been found to underlie several different ED. ED caused by *TP63* mutations include Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome (EEC; OMIM# 604292) [Celli et al., 1999], ADULT syndrome (OMIM# 103285) [Duijf et al., 2002], Limb-mammary syndrome (LMS; OMIM# 603543) [van Bokhoven et al., 2001], and Ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC or Hay Wells syndrome; OMIM# 106260) [McGrath et al., 2001]. A fifth condition caused by *TP63* mutations is Rapp-Hodgkin syndrome (OMIM# 129400) [Kantaputra et al., 2003]; however, this syndrome is now considered to represent the same clinical entity as AEC [Bertola et al., 2004; Clements et al., 2010]. Thus, in this manuscript, we will use the inclusive term “AEC” to refer to Hay Wells syndrome, Rapp-Hodgkin syndrome, or AEC syndrome.

ANKYLOBLEPHARON-ECTODERMAL DEFECTS-CLEFT LIP/PALATE (AEC) SYNDROME

A hallmark of the clinical AEC phenotype is the presence of severe scalp erosions (Fig. 1A). However, other skin sites may also be affected by erosions, including palms and soles (Fig. 1B). Skin erosions are a major cause of morbidity in infants with AEC, often complicated by local and systemic infections, which may be life-threatening [Vanderhooft et al., 1993; Siegfried et al., 2005; Julapalli et al., 2009]. Currently, there is no cure for the skin erosions, and symptomatic wound care is only partially effective [Julapalli et al., 2009].

In addition to the extensive skin erosions, the AEC phenotype often includes cleft lip, cleft palate, and abnormalities in several other ectoderm-derived tissues, including sweat glands, teeth, nails, limbs, and hair [Bree, 2009; Cole et al., 2009; Koster, 2010]. The observation that different appendages are affected in AEC patients reflects the crucial role for TP63 in the initial steps of forming all of these structures during development [Koster and Roop, 2004; Mikkola, 2007]. We chose to study the role of TP63 in the hair follicle in part because the regulatory pathways controlling normal hair follicle development and homeostasis have been relatively well-characterized [Schmidt-Ullrich and Paus, 2005; Duverger and Morasso, 2009], thus allowing us to link the effects of *TP63*-AEC mutations to specific signaling pathway abnormalities. Hair abnormalities in AEC patients include patchy or complete hair loss (alopecia), diminished hair density in areas where hair is present, and structural hair

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shaft abnormalities [Salinas and Montes, 1988; Hicks et al., 2001; Dishop et al., 2009] (Fig. 1C). Although sparse hair is a feature of most ED, the extensive and sometimes complete alopecia in AEC is likely a consequence of scarring as a result of scalp erosions. Systematic evaluation of AEC scalp biopsies and trichograms (i.e., microscopic analysis of plucked hair) would be valuable tools to help define the nature and causes of the alopecia, including the identification of the hair follicle cell types affected by TP63-AEC proteins. Research in our laboratories and others is currently addressing this question, and is expected to lead to a better understanding of the role of TP63 in hair follicle biology.

TP63 Mutations in AEC

The *TP63* gene encodes at least 10, and possibly more, transcription factors that differ only in their N- (TA and ΔN) and C-termini (α–ε) [Yang et al., 1998; McGrath et al., 2001; Koster, 2010]. Further, all known TP63 isoforms contain identical DNA binding and oligomerization domains. ΔNp63α is the predominantly expressed TP63 isoform in the epidermis and in skin appendages [Yang et al., 1998; Liefer et al., 2000; Koster et al., 2004; Laurikkala et al., 2006]. This isoform was also found to be critical for normal development and homeostasis of the skin in mice and humans [Mills et al., 1999; Yang et al., 1999; Koster and Roop, 2004; Koster and Roop, 2008].

TP63 mutations in AEC patients (*TP63*-AEC) primarily cluster in the α C-terminus of TP63, a domain that is encoded by exons 13 and 14 of the *TP63* gene and that contains a SAM domain (sterile α motif; a predicted protein-protein or protein-RNA interaction motif) [McGrath et al., 2001; Rinne et al., 2007, 2009]. In addition, mutations in the N-terminus of ΔNp63 isoforms have been described [Rinne et al., 2008], collectively suggesting a central role for mutant ΔNp63α isoforms (ΔNp63α-AEC) in the pathogenesis of AEC (Fig. 1D).

Cellular and Molecular Abnormalities in AEC Patient Skin

To gain insight into the pathological roles of TP63-AEC proteins, a first prerequisite is the identification of the cellular abnormalities that occur in the skin of AEC patients. To this end, we and others have analyzed skin biopsies from AEC patients. The overall consensus is that epidermal differentiation fails to occur normally in the skin of AEC patients. Importantly, these abnormalities were observed even when biopsies were obtained from non-lesional skin. At this time, the underlying reason for the focal nature of the skin erosions is not known; however, one possibility is that erosions are triggered by mechanical trauma.

In normal epidermis, proliferating cells are restricted to the basal layer and cells permanently withdraw from the cell cycle as they move suprabasally and embark on the terminal differentiation program [Koster and Roop, 2007]. This withdrawal from the cell cycle is, in part, mediated by the induction of IKKα and IRF6, two ΔNp63α target genes [Hu et al., 1999; Li et al., 1999; Takeda et al., 1999; Ingraham et al., 2006; Richardson et al., 2006; Koster et al., 2007; Marinari et al., 2008; Moretti et al., 2010]. Concomitant with the cell cycle withdrawal, markers of epidermal differentiation are induced, including keratins 1 and 10 (KRT1 and KRT10) and involucrin. Analysis of skin biopsies from AEC patients demonstrated abnormalities in epidermal differentiation as indicated by the persistence of proliferating cells in the suprabasal cell layers and the failure to induce KRT1, either

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completely or focally [McGrath et al., 2001; Marinari et al., 2008; Koster et al., 2009; Browne et al., 2011; Clements et al., 2012] (Fig. 2A,B).

In addition to impaired terminal differentiation, AEC patient skin shows abnormalities in the basement membrane zone [Koster et al., 2009; Clements et al., 2012]. These defects include aberrant and/or reduced synthesis of the basement membrane components collagen IV (COL4) [Koster et al., 2009] and collagen VII (COL7A1) [Clements et al., 2012] (Fig. 2C). Currently, it is not known whether these collagen genes are direct transcriptional targets of TP63. Further, expression of FRAS1, a basement membrane component that is under the direct transcriptional control of ΔNp63α, was reduced in AEC patients [Koster et al., 2007; Clements et al., 2012]. Collectively, these data indicate that expression of TP63-AEC proteins impairs epidermal differentiation and basement membrane integrity, two defects that likely contribute to the skin fragility observed in AEC patients.

INSIGHTS FROM CELL CULTURE STUDIES

The analysis of skin biopsies from AEC patients has led to important insights into the pathogenesis of AEC. However, a shortcoming of skin biopsies is that they represent a single point in time at which the disease phenotype has developed. Thus, these tissues do not allow one to distinguish between direct and indirect effects of TP63-AEC protein expression and how the disease develops over time. In order to obtain mechanistic insights into the disease mechanism, cell culture systems are a valuable tool.

For example, although AEC patient skin demonstrated increased TP63 protein expression, the underlying reason for this was not clear [Browne et al., 2011; McGrath et al., 2001; Clements et al., 2012]. Cell culture studies demonstrated that the stability of ΔNp63α-AEC proteins is increased compared to ΔNp63α-wt (wild type ΔNp63α) proteins, at least in part due to their inability to be appropriately targeted for ubiquitin- or phosphorylation-dependent degradation [Di Costanzo et al., 2009; Bellomaria et al., 2010; Browne et al., 2011]. In addition, an altered ability of TP63-AEC proteins to interact with other proteins in the cell may affect its ability to induce or repress TP63 target genes. Few proteins that differentially interact with TP63-AEC and TP63-wt proteins have been identified so far. Of these, SATB2 (special AT-rich binding protein-2) displays a stronger interaction with TP63-AEC than with TP63-wt proteins [Chung et al., 2011]. Due to this interaction, TP63-AEC proteins fail to induce PERP, a TP63 target gene that is required for epidermal cell adhesion and that is downregulated in a subset of AEC patients [Ihrie et al., 2005; Beaudry et al., 2009; Chung et al., 2011], thus potentially contributing to the tissue fragility observed in patient skin. Conversely, unlike TP63-wt proteins, TP63-AEC proteins cannot interact with the CCAAT-binding factor NF-Y [Testoni and Mantovani, 2006]. This in turn leads to a failure to repress Cyclin B2 (CCNB2) expression, a mechanism that may contribute to the suprabasal keratinocyte proliferation observed in AEC patient epidermis. Consistent with the findings that TP63-AEC proteins affect the expression of known TP63 target genes, reporter gene assays have demonstrated that TP63-AEC proteins have a dominant-negative effect towards TP63-wt proteins on the promoters of several target genes including IKKα [Marinari et al., 2008; Koster et al., 2009], Claudin 1 [Lopardo et al., 2008], KRT14 [Browne et al., 2011], and BPAG1 [Browne et al., 2011]. The latter observation raises the

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interesting possibility that structural or functional defects in hemidesmosomes (of which BPAG1 is a component) might contribute to the epidermal fragility observed in AEC patient skin. Collectively, data obtained from cell culture studies indicate that expression of TP63-AEC proteins leads to diminished expression of critical TP63 target genes involved in keratinocyte differentiation and tissue resistance to mechanical stress. However, whether, analogous to mutant TP53 proteins [Brosh and Rotter, 2009], TP63-AEC proteins also have gain-of-function effects could not be addressed in these studies. Unbiased transcriptome analysis of AEC cells is necessary to gain a more precise understanding of the molecular role of TP63-AEC proteins.

MODELING OF AEC

Mouse Models

To gain a better understanding of the pathological mechanism underlying AEC and the role of TP63-AEC proteins in the disease process, it is important to generate models that replicate the disease. Although skin biopsies from patients represent a biologically ideal model, they are difficult to obtain for rare disorders, yield patient cells with a limited life-span in vitro, and have a diverse genetic background. Mouse models offer an attractive alternative due to the availability of inbred stains in which all mice have an identical genetic background. Further, mice that are genetically engineered to carry *Trp63* mutations can be utilized to study the role of this gene in the development and maintenance of organs and tissues such as the skin.

To model human genetic disorders in mice, two main approaches have been undertaken. In the first model, a *TP63* mutation originally identified in AEC patients (L514F) was genetically engineered into the mouse genome. Mice heterozygous for this mutant allele developed clefting of the secondary palate, hypoplasia and blistering of the epidermis, and a delay in hair follicle morphogenesis [Ferone et al., 2012; Ferone et al., 2013]. Thus, some important features of AEC, such as cleft palate and hair follicle abnormalities, are replicated in this model. Importantly, however, the defective epidermal proliferation and hypoplasia are inconsistent with data obtained using skin biopsies from AEC patients [Marinari et al., 2008; Koster et al., 2009; Clements et al., 2012].

A second model generated by the Koster laboratory took advantage of data indicating that ΔNp63α-AEC proteins function, at least in part, as dominant-negative molecules. This would suggest that AEC keratinocytes, that is, keratinocytes carrying one *TP63*-AEC allele and one *TP63*-wt allele, exhibit impaired TP63 function. To test this hypothesis, we generated a mouse model that enables us to downregulate ΔNp63 expression in the epidermis of mice in a spatially and temporally controlled manner [Koster et al., 2007]. We observed that downregulating ΔNp63 in postnatal mouse epidermis led to skin erosions with histological characteristics similar to those observed in AEC patients [Koster et al., 2009]. Skin erosions in AEC patients and in our mouse model displayed impaired terminal differentiation, as demonstrated by the presence of proliferating cells in the suprabasal epidermis and the failure to induce markers of terminal differentiation such as Krt1 [Koster et al., 2007, 2009]. In addition, we observed basement membrane abnormalities including a discontinuous pattern of collagen IV synthesis, also observed in AEC patient skin [Koster et

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al., 2007, 2009]. Finally, we detected a reduced expression of those Trp63/TP63 target genes that are downregulated in AEC patient skin, including Fras1 and IKKα [Koster et al., 2007; Marinari et al., 2008; Clements et al., 2012]. Thus, in addition to the skin erosions, we have been able to successfully mimic critical cellular and molecular features of AEC patient skin in our mouse model, suggesting that this model will be a useful tool to understand AEC pathology.

Human Models

As outlined above, genetically engineered mouse models have provided important insights into the function of TP63 during skin development and homeostasis. Further, at least some aspects of AEC have been recapitulated in these model organisms. However, none of the mouse lines developed to date completely mimics the AEC skin phenotype at both the genetic and the phenotypic level. This observation might, at least in part, be due to intrinsic differences in the biology of mouse and human skin [Godin and Touitou, 2007; Wong et al., 2011]. Human cell-based disease models are thus required to fill the gap in our understanding of the molecular and cellular mechanisms that lead to skin erosions in AEC patients.

Cell culture-based systems have a lower complexity than animals, thus facilitating theidentification of molecular defects specific to a particular cell type, such as skin keratinocytes. For AEC, one approach to generate AEC tissue models has been to introduce *TP63*-AEC expression constructs into normal human keratinocytes via retroviral vectors [Zarnegar et al., 2012]. These keratinocytes were then used to generate skin equivalents in cell culture. A transcriptome analysis of these cells led to the identification of signaling pathways that link TP63-AEC to the aberrant expression of epidermal differentiation genes. Consistent with AEC patient skin, a reduction of KRT1 expression was observed in this model. A disadvantage of this system is that ratio of TP63-AEC and TP63-wt expression is likely different from that found in patients. Consequently, transcriptome changes caused by TP63-AEC expression in these cultures might not accurately reflect transcriptome changes in AEC patient skin. Another concern is that the type of cell culture system described above relies on cells isolated from discarded surgical material, that is, the genetic background of these cells is not known and may not be identical in each experiment. Importantly, such differences in genetic background can significantly affect disease phenotypes. For example, it is well-known that the same *TP63* mutation can lead to different clinical manifestations, even within individuals from the same family [Dianzani et al., 2003; Bertola et al., 2004; Clements et al., 2010].

As described in more detail below, technology developed in recent years now enables us to introduce or correct *TP63*-AEC mutations in the mammalian genome. Thus, we can now generate genetically matched pairs of cells that differ only with respect to the *TP63* gene (*TP63*-wt vs. *TP63*-AEC). Because gene expression variability as a result of differences in genetic background are effectively eliminated in these matched pairs, they are ideally suited to conduct transcriptome and signaling pathway analyses, crucial steps in elucidating the molecular pathology underlying AEC.

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INDUCED PLURIPOTENT STEM CELLS (IPSC) AS TOOLS TO STUDY AEC

Patient-derived cells are not only essential for elucidating disease mechanisms; they are also invaluable tools for screening and testing drugs aimed at correcting cellular defects (Fig. 3). However, AEC research has been hampered by the limited availability of patient cells. First, epidermal keratinocytes, the cell type affected in AEC skin, have a limited life span in vitro. Second, obtaining multiple skin biopsies of pediatric patients is not a desirable approach to obtain cells for large scale experiments.

Fortunately, the recent development of induced pluripotent stem cell (iPSC) technology has provided an elegant approach to circumvent these difficulties [Schambach et al., 2010; Selvaraj et al., 2010; Yamanaka and Blau, 2010; Hockemeyer et al., 2011] (Fig. 3). iPSC have properties similar to embryonic stem cells. They can be propagated indefinitely, they can be genetically modified, and they can be induced to differentiate into keratinocytes [Bilousova et al., 2011; Itoh et al., 2011; Tolar et al., 2011]. Consequently, iPSC technology can provide us with the unlimited source of genetically defined material necessary for disease studies and the development for new cell-based therapies for AEC (see below).

The first step in the generation of iPSC is the isolation of skin cells from AEC patients, either fibroblasts (the most common approach) or keratinocytes [Aasen et al., 2008; Polo et al., 2010; Tolar et al., 2011] (Fig. 5A). Amazingly, iPSC have also been generated using plucked human hair [Aasen and Izpisua Belmonte, 2010]. Primary patient cells are then transduced with genes encoding so-called reprogramming factors, which convert these cells into iPSC [Yamanaka, 2008]. A critical issue at this step is the choice of a vector system to introduce these factors. Retroviral and lentiviral vector systems have been widely used due to their efficiency in transducing cells. However, because these vectors integrate into the genome, they induce mutations, an undesired side effect. Other non DNA-integrating vector systems, such as plasmids, in vitro transcribed RNA, or proteins [Qin et al., 2009; Carey et al., 2009; Yakubov et al., 2010; Lai et al., 2011] have successfully been used, but are either very inefficient in generating iPSC or are technically difficult to use. New vector systems are thus needed, especially if iPSC are to be used for human therapy. For research purposes, the non-integrating but highly effective Sendai virus vectors that we routinely use to derive iPSC from AEC patient samples, appear to be ideal [Fusaki et al., 2009] (Fig. 5B).

Editing the iPSC Genome

As outlined above, correcting *TP63-AEC* mutations in iPSC will provide us with the opportunity to generate matched pairs of iPSC-derived keratinocytes that differ only in their *TP63* status. Recent advances in mammalian genome editing make it possible to accomplish this gene correction in an efficient way. Central to these approaches are sequence-specific DNA nucleases that target the *TP63* gene locus. Both zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) have been shown to be effective in genetically manipulating the iPSC genome [Zou et al., 2009; Hargus et al., 2010; Hockemeyer et al., 2011; Miller et al., 2011].

The TALEN shown in Figure 4 were designed to target the *TP63* gene close to a cluster of *TP63*-AEC mutations that occur in our cohort of AEC patients. Two “half” (enzymatically

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inactive) TALEN are designed which bind adjacent DNA segments. Because correct binding of two TALEN components is required to generate an active nuclease, the specificity of gene targeting using this system is very high. Upon correct dimerization, the active nuclease generates a double strand (ds) DNA break, which facilitates homologous recombination between the *TP63* locus and gene targeting vectors (also referred to as exchange matrices), which are introduced into the cells together with the TALEN (Fig. 4). A gene targeting vector carrying wild type *TP63* sequences can thus be used to correct *TP63*-AEC mutations. Because *TP63* mutations in AEC cluster in the same region of the gene, a single pair of TALEN can be used to repair several different *TP63*-AEC mutations. This represents a tremendous advantage of this technology over other methods, and is important for the development of effective cell therapies to treat skin erosions in AEC patients (see below).

BUILDING PATIENT-DERIVED SKIN IN VITRO

Human iPSC can be induced to differentiate into the ectodermal lineage. The required cell culture protocols were originally developed for embryonic stem cells [Aberdam et al., 2008; Metallo et al., 2008] and later adapted for iPSC [Bilousova et al., 2011; Itoh et al., 2011; Tolar et al., 2011]. Essentially, iPSC are exposed to combinations of factors such as retinoic acid, BMP4, or ascorbic acid. As a result, the cells begin to express epithelial markers such as keratins 8 and 18 (KRT8, KRT18) before synthesizing TP63, KRT14, and other keratinocyte markers. After 30 days of in vitro differentiation, a pure population of iPSC-derived keratinocytes can then be isolated via fluorescence activated cell sorting (FACS; Fig. 5C,D). Keratinocyte surface markers such as α6 integrin and β4 integrin are useful markers for FACS isolation of keratinocytes.

Besides conventional 2D cell culture (culture of keratinocytes on collagen IV-coated plastic dishes), different methods to generate in vitro skin equivalents can be applied to such FACS-sorted keratinocytes. For example, these cells can form an epidermis-like structure on artificial extracellular matrix (ECM) substitutes (Fig. 6A). Alternatively, fibroblasts embedded into a collagen matrix can be used as a substrate for these cells to form epidermis-like 3D cultures [Simpson et al., 2010] (Fig. 6B). The ultimate approach to generate skin equivalents is a xeno-transplant onto immunodeficient mice, for example, by grafting a mixture of fibroblasts and keratinocytes onto mice using a silicone grafting chamber [Lichti et al., 2008]. The latter approach connects the newly formed tissue to the blood circulation of a mammal, a step closer to mimicking true skin biology.

Each of the systems described above has distinct advantages and disadvantages. Simple 2D cell cultures consist of a rather homogeneous population of keratinocytes under culture conditions that suppress differentiation. These cultures are considered a model for undifferentiated keratinocytes, such as those found in the basal layer of the epidermis. Since basal keratinocytes express high levels of TP63, such 2D cultures are well-suited for characterizing cell autonomous functions of TP63. Three dimensional culture systems (skin equivalents) are ideally suited to test the effects of *TP63*-AEC mutations on keratinocyte differentiation. Given that skin of AEC patients is characterized by severe epidermal differentiation defects, these in vitro skin equivalents are important tools to replicate and thus analyze the AEC disease phenotype in vitro. Lastly, the recent development of xeno-

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transplant technology that allows for the generation of hair follicles using iPSC-derived human keratinocytes and mouse dermal papilla cells [Veraitch et al., 2013] opens up the possibility to replicate the hair defects observed in AEC in an animal transplant model using human keratinocytes, thus providing us with new tools to understand the underlying pathology and to develop new treatment options.

INVESTIGATING THE DISEASE MECHANISM

A few genes affected in their expression by *TP63*-AEC mutations have been identified [Marinari et al., 2008; Koster et al., 2009; Moretti et al., 2010; Clements et al., 2012; Sen et al., 2012; Zarnegar et al., 2012]. However, we currently do not have a comprehensive understanding of the molecular and cellular pathways affected by TP63-AEC proteins. Identifying molecular mechanisms will not only advance our understanding of *TP63*-AEC function, it might also lead to the identification of potential therapeutic targets for the treatment of skin erosions in AEC patients. A first step towards this goal will be a comparative transcriptome analysis of cells expressing *TP63*-AEC and *TP63*-wt. Conisogenic cell systems (i.e., genetically matched pairs of cells that differ only in the *TP63* gene), will be of particular importance since their use will minimize genetic background effects on gene expression. To this end, patient iPSC in which the *TP63* mutation has been repaired (see above) and the original patient iPSC will be differentiated into keratinocytes. Besides transcriptome analysis and promoter binding studies, proteomic studies will be required to understand how TP63-AEC and TP63-wt proteins function in these cells. In addition, protein stability, the ratio of TP63-AEC and TP63-wt proteins, as well as the ability of TP63-AEC to contribute to multimeric protein complexes will be assessed. All of these data combined will yield a more complete picture of the disease process and will enable us to characterize the pathological mechanisms underlying AEC. Importantly, similar approaches can be used for modeling and characterizing of other *TP63*-related ED, such as EEC. Further, a comparative transcriptome analysis of different ED caused by *TP63* mutations will significantly advance our understanding of TP63 function both in normal development and in disease.

OUTLOOK—STEM CELL BASED THERAPIES

There is currently no cure or effective treatment for skin erosions that occur in AEC patients. iPSC technology holds the promise to generate genetically corrected autologous keratinocytes with a low likelihood of rejection. However, further characterization of the immune responses to these transplanted keratinocytes should be a focus of future research [Pick et al., 2009; Apostolou and Hochedlinger, 2011; Araki et al., 2013]. Further, iPSC derived for therapy must be free of viral vectors, which means that more efficient and safe reprogramming methods still need to be developed. Another concern is the potential of accumulating mutations in iPSC, either because patient cells that already carry mutations are converted into iPSC or because prolonged cell culture has the potential to generate mutations [Lowry and Quan, 2010; Mayshar et al., 2010; Gore et al., 2011].

However, it is likely that these remaining technical challenges will be overcome. iPSC technology offers a unique opportunity to both elucidate the molecular and cellular

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mechanisms underlying AEC and to eventually develop novel methods to treat the skin manifestations of this disorder.

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Abbreviations

ED	ectodermal dysplasia
AEC	ankyloblepharon-ectodermal defects-cleft lip/palate syndrome
TP63-AEC	TP63 containing a mutation identified in AEC patients
iPSC	induced pluripotent stem cell
TALEN	transcription activator-like effector nucleases
FACS	fluorescence activated cell sorting

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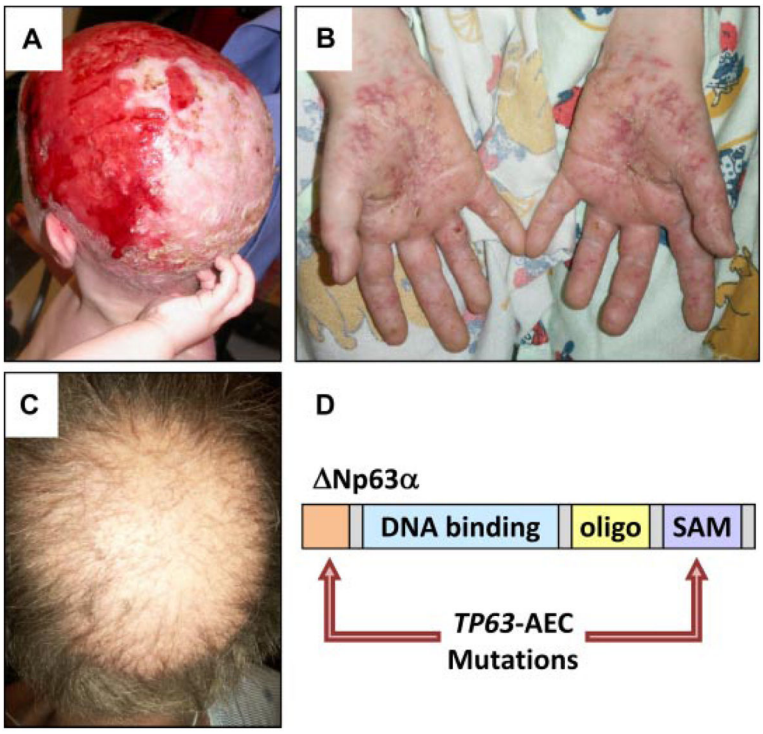

FIG. 1.

Mutations in *TP63* underlie AEC, characterized by skin fragility and hair abnormalities. A: Scalp erosions on an AEC patient. B: Palmar epidermal erosions. C: Hair abnormalities, including partial alopecia, reduced hair shaft density, and hair shaft abnormalities. D: Schematic of ΔNp63α indicating the most common mutation sites identified in AEC patients.

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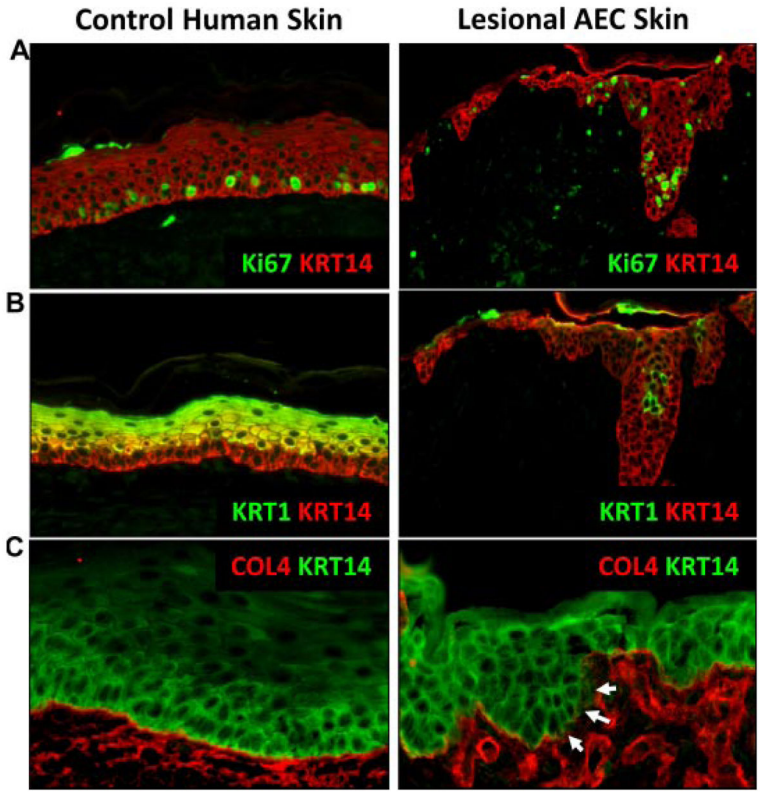

FIG. 2.

Cellular abnormalities observed in the skin of AEC patients. A: Proliferation, as shown here by Ki67 immunofluorescent staining (green) is restricted to the basal layer in normal human skin, but extends suprabasally in AEC patient skin. B: The marker for terminal differentiation, KRT1 (green/yellow), is induced in the first suprabasal layer in control human skin, but is delayed in its expression or absent from AEC patient skin. C: Immunofluorescent staining for the basement membrane component Collagen IV (COL4; red) shows uninterrupted staining along the basement membrane in control human skin, but is disrupted in AEC patient skin. Immunofluorescent staining for keratin 14 (KRT14) was used to highlight the epidermis in A (red), B (red), and C (green). Image reproduced from [Koster et al., 2009].

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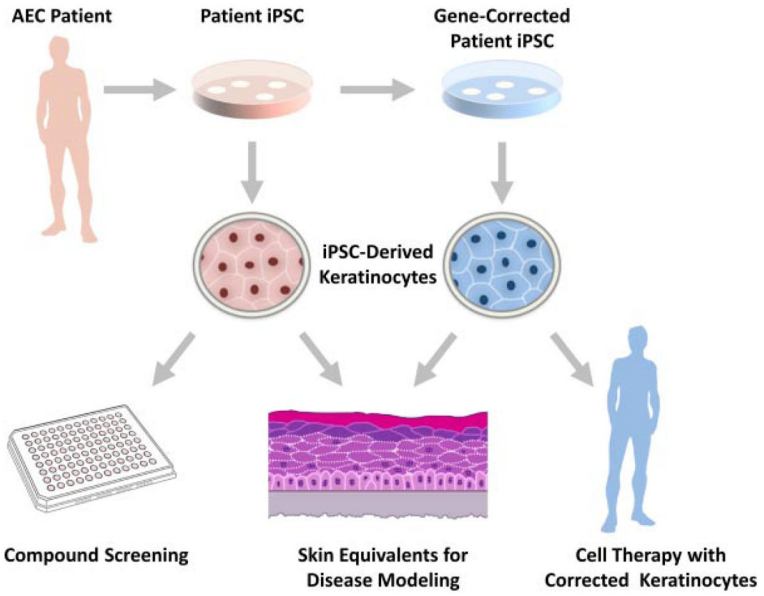

FIG. 3.

Disease models and new approaches to develop effective AEC therapies. Cells derived from patient skin biopsies are converted into iPSC. Using genome editing tools such as TALEN, it is possible to correct the *TP63*-AEC mutation, leading to the generation of conisogenic cell lines that differ only with respect to the status of the *TP63* gene (*TP63*-AEC, *TP63*-wt). These cells can be differentiated into keratinocytes which will be useful tools to model the disease in 3D cultures, to test compounds for therapeutic effects on mutant keratinocytes, and to develop cell-based therapies (transplantation of gene-corrected keratinocytes onto the patient).

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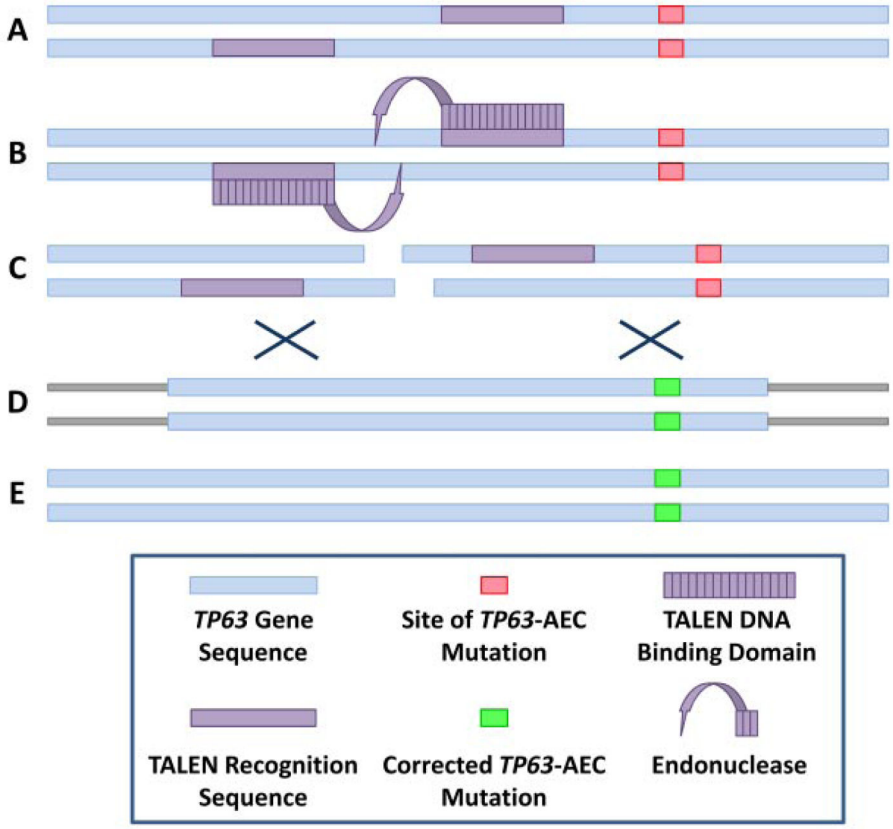

FIG. 4.

TALEN as tools to correct *TP63*-AEC mutations. A: Schematic representation of the *TP63*-AEC allele carrying a mutation (pink box). B: Two half TALEN bind to target sequences in the gene (purple boxes). Binding of these proteins leads to dimerization and activation of a DNA endonuclease activity. C: Double-stranded break in the *TP63* DNA sequence. D: Homologous recombination of the *TP63*-AEC locus with a targeting vector carrying *TP63* wild type sequences (green box). E: *TP63* locus after correction of the AEC mutation.

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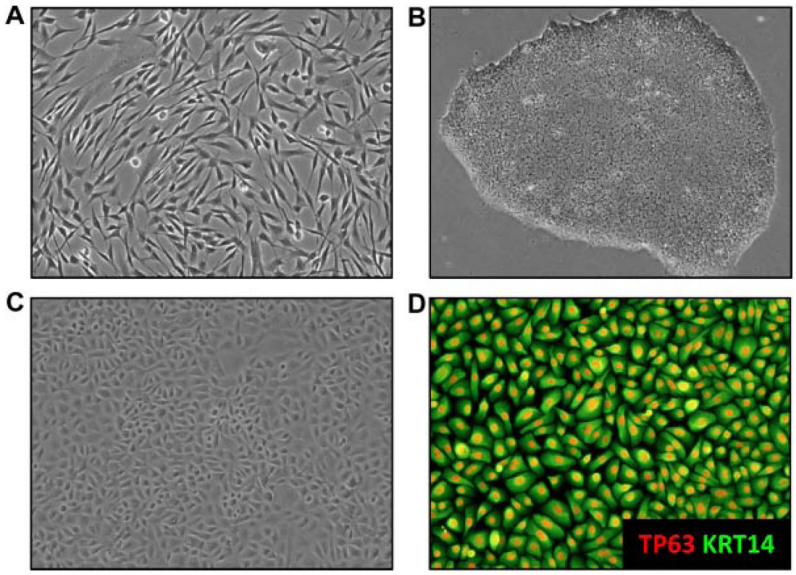

FIG. 5.

Generation of iPSC-derived keratinocytes. A: Fibroblasts isolated from a skin biopsy of a pediatric AEC patient were transduced with reprogramming factors. B: Example of an iPSC colony derived from patient fibroblasts. Exposure of the iPSC to differentiation factors such as BMP4, retinoic acid, or ascorbic acid induces the development of ectodermal precursor cells. C: Further differentiation of these cells leads to the development of cells with a typical keratinocyte morphology. D: FACS sorting using antibodies against keratinocyte markers such as α6 integrin and β4 integrin leads to the isolation of a pure population of cells homogeneously expressing the keratinocyte markers TP63 (red) and keratin 14 (KRT14; green).

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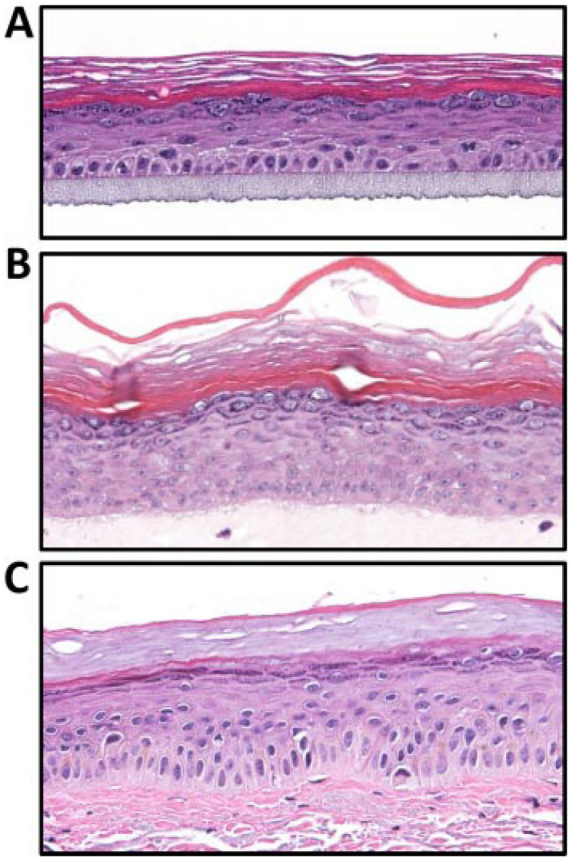

FIG. 6.

Examples of in vitro culture systems used to generate AEC models. Monolayer cultures of keratinocytes are transferred to an extracellular matrix allowing for the development of a 3D epidermal structure. A: Three dimensional culture generated on an artificial extracellular matrix. B: Three dimensional culture generated on a collagen plug with embedded fibroblasts. C: Example of normal human skin. Note the histological similarity of in vitro generated skin equivalents and normal human skin.