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Use of Induced Pluripotent Stem Cells in Dermatological Research

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Induced pluripotent stem cells (iPSCs) have the potential to differentiate into any cell type of the body. iPSCs are generated through a process termed “reprogramming,” which entails the introduction of a set of transcription factors into somatic cells, such as dermal fibroblasts. iPSC clones, recognizable by their morphology (Figure 1a), arise from these cultures, usually within 14–21 days. In addition to their pluripotency, another important characteristic of iPSCs is that they can be propagated in cell culture indefinitely. Thus, an unlimited supply of iPSCs can be generated from a small skin biopsy. These iPSCs can then be differentiated into different types of somatic cells. For example, iPSCs can be directed to differentiate into epidermal keratinocytes, one of the cell types often affected in skin disorders. iPSC-derived keratinocytes can then be used to generate a stratified epidermis, either *in vitro* (3D skin equivalent cultures) or *in vivo* (xenotransplantation of keratinocytes onto immunodeficient mice; see Koch *et al*., 2014; Koster *et al*., 2014 for references). The ability to generate unlimited numbers of disease-specific keratinocytes provides an ideal tool for basic scientists to explore the molecular mechanisms underlying different skin disorders. Further, recently developed technologies now enable investigators to correct disease-causing mutations in iPSCs. These cells could then be used to generate gene-corrected, healthy

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CONFLICT OF INTEREST

The authors state no conflict of interest.

CME ACCREDITATION

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SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for teaching purposes is available at http://dx.doi.org/10.1038/jid.2014.238.

replacement skin for patients affected by genetic skin disorders. A major advantage of this approach is that patients would be treated with cells that are unlikely to be immunologically rejected.

iPSC GENERATION AND CHARACTERIZATION

iPSCs are most frequently generated from fibroblasts obtained from a small (e.g., 4-mm) skin biopsy. A set of cDNAs representing four specific genes ("Yamanaka factors")—*POU5F1* (*Oct3/4* in the mouse), *SOX2*, *KLF4*, and *MYC* (Takahashi and Yamanaka, 2006)—is then introduced into the cells to induce pluripotency. Initially, these reprogramming factors were introduced into cells using retroviral vectors. Although successful, retroviruses integrate into the host genome, thus generating mutations. To circumvent this problem, nonintegrative systems have been developed to introduce reprogramming factors into cells such as plasmids, proteins fused to cell-penetrating peptides, mRNAs, and nonintegrating Sendai virus vectors (reviewed in Schambach *et al*., 2010).

After the reprogramming factors are introduced, cells with morphological characteristics similar to those of embryonic stem cells (ESCs) arise (Figure 1a). To determine whether successful iPSC conversion has occurred, these putative iPSC colonies are evaluated for the expression of genes associated with pluripotency, such as *NANOG*, *SSEA-3*, and *TRA1-60* (Figures 1b–d; see also references in Tolar *et al*., 2013). Further, the absence of gross genetic abnormalities is assessed by karyotype analysis (i.e., a microscopic evaluation of chromosome numbers and structures). Finally, proof of pluripotency is obtained by assessing the ability of iPSCs to differentiate into cell types representing all three germ layers (endoderm, ectoderm, and mesoderm) *in vitro* or *in vivo*. For example, intramuscular or subcutaneous injection of human pluripotent iPSCs into immune-compromised mice will give rise to teratomas, tumors composed of cells representing each of the three germ layers (Figure 2; Tolar *et al*., 2013).

GENERATING KERATINOCYTES FROM iPSCs

Treatment of iPSCs with retinoic acid and BMP4 in cell culture directs the differentiation of these stem cells into keratinocytes (Figure 1e) (Itoh *et al*., 2011; Petrova *et al*., 2014). These cells express well-established keratinocyte markers such as KRT14 and TP63 (Figure 1f), and they can be isolated as pure cell populations using fluorescence-activated cell sorting with antibodies against keratinocyte cell surface markers such as ITGA6 and ITGB4. Further, iPSC-derived keratinocytes undergo terminal differentiation upon calcium exposure, as demonstrated by the expression of keratinocyte differentiation markers such as KRT1 and loricrin. Finally, iPSC-derived keratinocytes can form a fully stratified epidermis either *in vitro* (Figures 1g and 1h) or when transplanted onto immunodeficient mice. In addition to keratinocytes, other components of human skin, such as melanocytes and fibroblasts, can also be generated from iPSCs (Ohta *et al*., 2011; Itoh *et al*., 2013). By combining the three main cellular components of human skin, namely keratinocytes, fibroblasts, and melanocytes, it should soon be possible to regenerate fully functional human skin.

Beside generating epidermis, iPSC technology can be used to generate skin appendages. For example, two groups demonstrated that human iPSC-derived ectodermal precursor cells (EPCs) can contribute to the formation of hair follicles *in vivo* (Figure 3; Veraitch *et al*., 2013; Yang *et al*., 2014). In both studies, human iPSC-derived EPCs were combined with trichogenic neonatal mouse dermal papilla cells and then transplanted into immunodeficient mice. Several weeks later, newly generated hair follicles were observed that were, in part, derived from the human EPCs. The tremendous advances made in the past two years in this field of research suggest that we should soon be able to generate all major components of human skin in the laboratory.

APPLICATION OF iPSC TECHNOLOGY IN RESEARCH AND THERAPY

One defining property of iPSCs is their ability to be expanded indefinitely. The essentially limitless amount of resulting material now enables skin researchers to generate human models for various genetic skin disorders (reviewed in Koch *et al*., 2014; Koster *et al*., 2014). These models are used not only to gain a better understanding of pathological mechanisms responsible for various skin disorders but also for the generation of cell-based screening systems designed to identify compounds that reverse or diminish disease phenotypes (Figure 4).

One therapeutic goal is to utilize iPSC technology to generate genetically corrected keratinocytes from patients affected by genetic skin disorders. For example, Tolar and colleagues (Tolar *et al*., 2013) recently demonstrated that gene-corrected iPSCs can be generated from the skin of patients with a mosaic form of recessive dystrophic epidermolysis bullosa (RDEB). RDEB is caused by mutations in *COL7A1*, the gene encoding collagen VII. These mutations prevent the synthesis of sufficient amounts of collagen VII, leading to skin blistering. The patient described by Tolar and colleagues exhibited patches of normal-appearing skin in which the *COL7A1* gene was spontaneously corrected (Figure 5a and b). By generating iPSCs and, subsequently, iPSC-derived keratinocytes from these patches (Figure 5e), the authors were able to provide proof of principle that iPSC technology can be used to generate essentially unlimited amounts of clinically normal epidermis from patients with a mosaic form of RDEB.

Despite its potential use for patients with mosaic forms of skin disorders, this approach is not applicable to patients with nonmosaic skin disorders. For the latter group of skin disorders, genetic mutations must be corrected *in vitro* to generate healthy replacement skin. This can be accomplished using sequence-specific DNA nucleases (e.g., TALE nucleases; Miller *et al*., 2011) designed to cut specific DNA sequences. If these nucleases are simultaneously introduced into patient-derived cells with a plasmid containing the corrected DNA sequence, homologous recombination leads to the repair of disease-causing point mutations (see references in Koch *et al*., 2014; Koster *et al*., 2014). The resulting gene-corrected iPSCs constitute an ideal source for generating unlimited supplies of patient-specific (and therefore most likely immunologically well-tolerated), healthy skin grafts. These gene corrections can be done either in iPSCs or in primary patient cells. For example, Osborn and colleagues (Osborn *et al*., 2013) recently corrected an RDEB-causing *COL7A1* mutation in patient fibroblasts. These fibroblasts were then turned into iPSCs and

subsequently into keratinocytes expressing collagen VII, suggesting that this technology could indeed be used to treat genodermatoses with healthy (gene-corrected) patient-derived replacement tissue.

SUMMARY AND CONCLUSIONS

iPSCs combined with gene-editing technologies are poised to have a significant impact on our ability to generate *in vitro* and *in vivo* disease models for genodermatoses caused by single point mutations. Generating keratinocytes that are genetically identical except for the presence or absence of a disease-causing mutation will provide researchers with ideal systems to assess defects in iPSC-derived patient keratinocytes at the RNA, protein, and functional levels. Further, this approach will enable us to develop patient cell–based screening systems to identify compounds capable of correcting defects in patient keratinocytes. In the long term, this technology may also be used to generate patient-derived, gene-corrected skin that could be transplanted onto patients from whom the original iPSCs were derived. Thus, this may lead to the development of novel therapies for debilitating genetic skin diseases, such as skin blistering or skin fragility disorders, for which no current therapies exist.

Although the research potential of iPSCs is without doubt significant, there are still concerns regarding the safety of using this technology for patient care. For example, introduction of undifferentiated iPSCs into patients could lead to the formation of teratomas. Further, prolonged culture has the potential to introduce mutations into the iPSC genome. To overcome these concerns, methods are under development that allow for the generation of pure populations of target cells, such as keratinocytes, that do not contain undifferentiated iPSCs. Further, approaches such as deep sequencing can be utilized to identify mutations in iPSCs before they are used therapeutically. Finally, the generation and genetic manipulation of iPSCs require the introduction of recombinant DNA into these cells. Efficient methods to introduce DNA into cells without leaving a genetic footprint are required. Nevertheless, the clinical use of iPSC-based technology is rapidly approaching, as demonstrated by a clinical trial at the RIKEN Center for Developmental Biology in Japan, in which patients will be treated with iPSC-derived retinal pigment epithelium cells for age-related macular degeneration (Cyranoski, 2013).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ADVANTAGES

- iPSCs can be generated from skin biopsies.
- iPSCs can be propagated indefinitely *in vitro*, providing a renewable source of cells.
- iPSCs can be differentiated into any cell type.
- Cells differentiated from iPSCs can be used for disease modeling and drug screening.
- Disease-causing mutations can be corrected in iPSCs.
- iPSC-derived cells, such as keratinocytes, can be used in cell therapy.
- Generation of iPSCs involves the use of adult cells, bypassing ethical concerns associated with using embryonic stem cells (ESCs).

LIMITATIONS

- iPSCs can acquire genetic mutations during reprogramming or *in vitro* culture.
- Undifferentiated iPSCs, if transferred to a patient, could form tumors (teratomas).
- More stringent protocols must be developed and standardized for the generation, maintenance, characterization, and differentiation of iPSCs before they can be considered a viable option for clinical use.

QUESTIONS

This article has been approved for 1 hour of Category 1 CME credit. To take the quiz, with or without CME credit, follow the link under the "CME ACCREDITATION" heading.

1. How are iPSCs generated?
   - **A.** Fusion between a somatic cell and embryonic stem cell.
   - **B.** Transplantation of a nucleus from a somatic cell into an enucleated egg.
   - **C.** Collection of cells from the inner cell mass of a blastocyst.
   - **D.** Ectopic expression of reprogramming factors converting somatic cells to stem cells.
2. What are some criteria used to define iPSCs?
   - **A.** ESC-like cellular and colony morphology.
   - **B.** Ability to be expanded indefinitely.
   - **C.** Ability to differentiate into different cell types.
   - **D.** All of the above.
3. List a main advantage of iPSC technology.
   - **A.** Cells differentiated from iPSCs are unlikely to be immunologically rejected when used in autologous cell-replacement therapies.
   - **B.** iPSCs are not tumorigenic and do not form teratomas.
   - **C.** iPSCs are easier to maintain in culture than ESCs.
   - **D.** iPSCs differentiate more efficiently than ESCs.
4. What is one potential use for iPSCs?
   - **A.** iPSCs can prevent tumor formation.
   - **B.** iPSCs can be used to cure everything.
   - **C.** iPSC-derived cells can be used to screen for novel compounds for the treatment of specific disorders.
   - **D.** iPSCs can be used effectively when added to antiaging skin creams.
5. What is an issue of concern when considering iPSCs for use in clinical applications?
   - **A.** Gene correction cannot be performed in iPSCs.
   - **B.** iPSCs may acquire genetic mutations while being expanded *in vitro*.
   - **C.** Cells differentiated from iPSCs may outcompete host cells of the same type after transplantation.

D. After a period of time, cells differentiated from iPSCs revert to their pluripotent state.

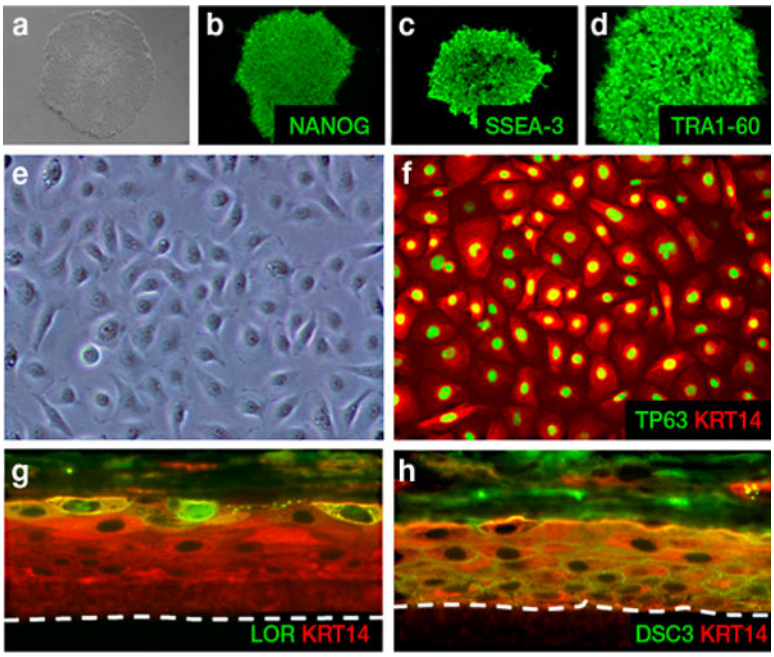

Figure 1. Generation of induced pluripotent stem cells (iPSCs) and iPSC-derived keratinocytes

(**a**) human iPSC colony. (**b–d**) Immunofluorescence microscopy of iPSC colonies with antibodies against pluripotency markers demonstrating that the iPSCs have the typical characteristics of pluripotent stem cells (**b**, NANOG; **c**, SSEA-3; **d**, TRA1-60). (**e**) Micrograph of an iPSC-derived keratinocyte culture that (**f**) expresses KRT14 (red) and TP63 (green), two marker proteins for keratinocytes. (**g, h**) 3D skin equivalent generated from human iPSC-derived keratinocytes. The sections were stained with antibodies against (**g**) LOR (loricrin; green) and KRT14 (red) and (**h**) DSC3 (desmocollin 3; green) and KRT14 (red), proteins expressed in normal human epidermis.

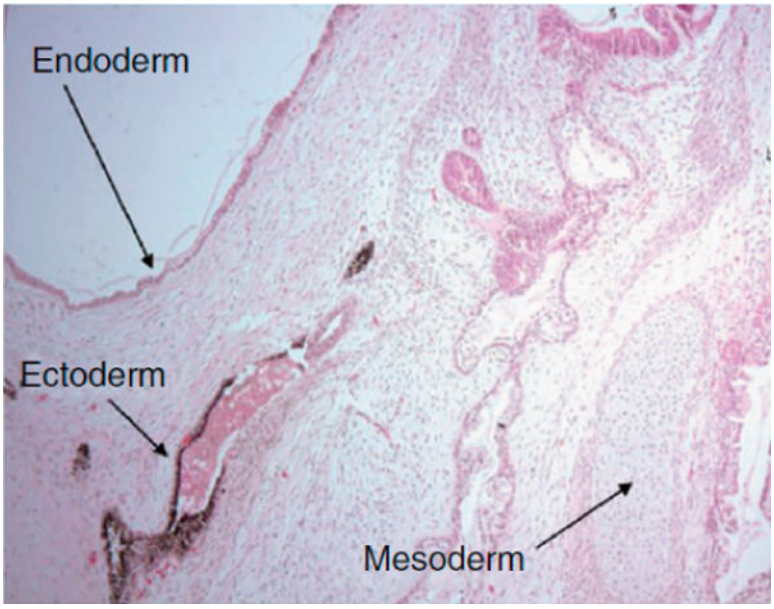

Figure 2. Induced pluripotent stem cell (iPSC)-induced teratoma in an immunodeficient mouse
Note that the iPSCs differentiated into cells representing mesodermal, ectodermal, and endodermal lineages. Reprinted with permission from Tolar *et al*., 2013.

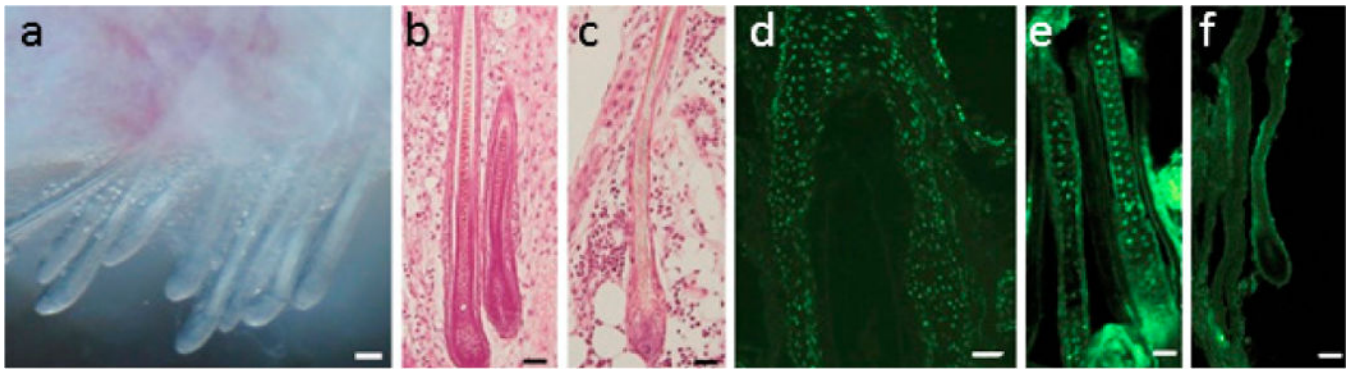

Figure 3. Generation of skin appendages with induced pluripotent stem cell (iPSC)-derived ectodermal precursor cells

(**a**) Morphology of hair follicles (HFs) formed in areas transplanted with human iPSC-derived ectodermal precursor cells (hiPSC-EPCs). (**b**) Pilosebaceous unit (HF and sebaceous gland) formed with participation of hiPSC-EPCs. (**c**) Normal mouse HF control. (**d–f**) Staining of HF with a human-specific antibody. (**d**) Human HF and (**e**) hiPSC-EPC-derived HF stain positive, whereas the mouse HF (**f**) is negative. Bars = 50 mm. Reprinted with permission from Veraitch *et al*., 2013.

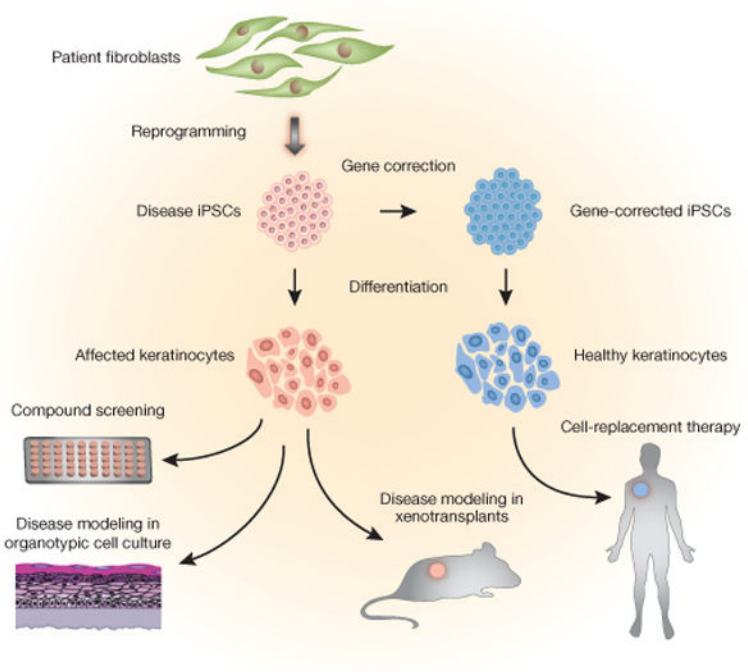

Figure 4. Outline of the basic principles underlying induced pluripotent stem cell (iPSC) technology as it is used for disease modeling and for the generation of replacement tissue in regenerative medicine

Somatic cells (e.g., biopsy-derived fibroblasts) are reprogrammed into iPSCs. Using genome-editing tools, such as TALE nucleases, point mutations can be corrected in the iPSCs (or in the original fibroblasts; see text for details). iPSCs can be differentiated into keratinocytes. Gene-corrected (control) and clinically affected keratinocytes can then be used in several experimental approaches: to set up compound screening to identify drugs interfering with disease phenotypes or to model clinical skin phenotypes *in vitro* (organotypic culture) or *in vivo* (generation of a human epidermis in immunodeficient mice, xenotransplants). Finally, gene-corrected keratinocytes can be used to produce replacement tissue for patients, for example, for patients with genetic blistering diseases (e.g., recessive dystrophic epidermolysis bullosa, see text for details).

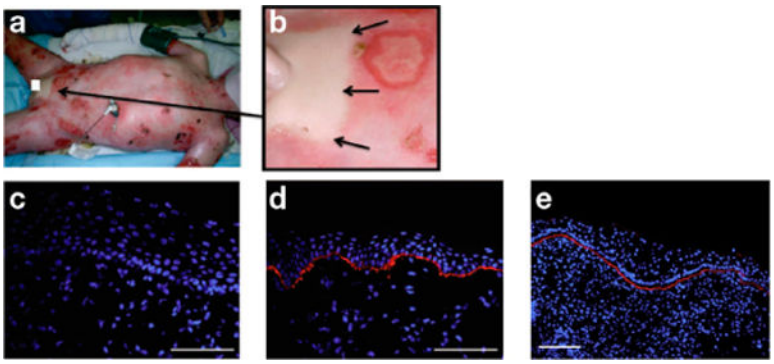

Figure 5. Generation of phenotypically normal keratinocytes from patients affected by a mosaic form of recessive dystrophic epidermolysis bullosa (RDEB) using induced pluripotent stem cell (iPSC) technology

(**a**) Child with a mosaic form of generalized severe RDEB. The arrow points to a clinically normal skin area shown at higher magnification in (**b**). (**c**) Immunofluorescence microscopy demonstrates the absence of collagen VII staining in the affected skin of the patient, whereas (**d**) clinically normal areas show collagen VII at the epidermal–dermal junction. (**e**) iPSC-derived teratoma containing an epidermal-like structure expressing collagen VII at the dermal–epidermal junction, demonstrating that the iPSC-derived keratinocytes that originated from cells in the clinically normal skin of the patient produce collagen VII (collagen VII staining in red, nuclei are stained in blue). Bars = 50 μm. Reprinted with permission from Tolar *et al*., 2013.