Promise of Human Induced Pluripotent Stem Cells in Skin Regeneration and Investigation

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Induced pluripotent stem cell (iPSC) technology has initiated a new era in biomedical science. The skin has been realized as an ideal platform for iPSC applications; unlike other organs, the skin is easily accessible, highly proliferative, and reconstitutable. Currently, skin equivalents can be generated from iPSCs not only from healthy individuals but also from patients with genodermatoses, providing novel platforms for dissecting disease pathophysiology and establishing cell-based therapy. With their developmental plasticity, iPSCs may also enable the regeneration of skin appendages. The iPSC technology may provide novel remedies for intractable disorders, once key issues particularly, safety concerns, are cleared.

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INTRODUCTION

Human induced pluripotent stem cells (hiPSCs) hold great promise as material for regenerative medicine because of their unlimited proliferative capacity, mutipotency to differentiate into multiple lineages and, importantly, ethical acceptability (Uitto, 2011; Okano *et al.*, 2013). The significance of hiPSCs is especially highlighted in the development of cell-based remedies for intrinsically less proliferative organs, represented by the central nervous system, where induction of tissue stem/progenitor cells from undifferentiated stem cells is necessary (Nakamura and Okano, 2013; Okano *et al.*, 2013). In contrast to central nervous system, the skin and its appendages are endowed with high regenerative capacity enabling their continuous self-renewal (Cotsarelis, 2006; Blanpain and Fuchs, 2009). Putative epithelial and dermal stem cell populations have been identified and isolated as living cells from human skin (Barrandon and Green, 1987; Li *et al.*, 1998; Jahoda *et al.*, 2003; Toma *et al.*, 2005; Jensen and Watt, 2006; Ohyama *et al.*, 2006; Kuroda *et al.*, 2010). Methodologies for *in vitro* expansion of human keratinocytes (KCs) and fibroblasts have been established and three-dimensional human skin equivalent with epidermal and dermal layers is readily available for severe burn patients (Green *et al.*, 1979; Pellegrini *et al.*, 1999). However, major obstacles still remain, which can be achieved with the use of hiPSCs. Drawing some parallels with recent advances in iPSC research for central nervous system regeneration for spinal cord injury (SCI; Nori *et al.*, 2011; Kobayashi *et al.*, 2012; Nakamura and Okano, 2013; Okano *et al.*, 2013), the major focus of iPSC research in our institution, we attempted to delineate substantial benefits of using hiPSCs in investigative and clinical dermatology.

iPSC TECHNOLOGY OPENS A NEW ERA OF MEDICAL SCIENCE

The seminal discovery by Yamanaka and Takahashi that dermal fibroblasts can be directly reprogrammed into a pluripotent stage analogous to that of embryonic stem (ES) cells by the introduction of only four transcription factors (Klf4, Oct4, Sox2, and c-Myc) has greatly impacted regenerative medicine (Takahashi and Yamanaka, 2006; Takahashi *et al.*, 2007). Within a short time period, variety of cell types of multiple species, including human and non-human primates, has been successfully dedifferentiated into iPSCs (Uitto, 2011; Harding *et al.*, 2013). In addition to ES cell-like unlimited proliferative capacities, iPSCs possess pluripotency to differentiate into all three germ layers and contribute to various tissues (Takahashi and Yamanaka, 2006; Takahashi *et al.*, 2007). Theoretically, iPSCs can be artificially differentiated into desired terminally differentiated cells *in vitro*, when exposed to culture conditions appropriately directing their fate. Indeed, well-differentiated and functional cell populations, such as neural cells (Nori *et al.*, 2011), cardiomyoctes (Kattman *et al.*, 2011), and hematopoietic stem cells (Amabile *et al.*, 2013) have been generated from hiPSCs. The hiPSCs can

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This work was done in Tokyo, Japan.

Abbreviations: EB, epidermolysis bullosa; ES, embryonic stem; hiPSC, human induced pluripotent stem cell; iPSC, induced pluripotent stem cell; KC, keratinocyte; NS/PC, neural stem/progenitor cell; SCI, spinal cord injury

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be obtained from any individuals and are free from ethical issues accompanying human ES cells in using the human early embryos for their establishment. Cell populations that can hardly be prepared from adult tissue, for instance neural stem/progenitor cells (NS/PCs), can be induced from iPSCs (Nakamura and Okano, 2013). Thus, iPSCs provide immense opportunities for investigations of inaccessible human tissue, patient-specific cell therapies, and tissue engineering (Okano *et al.*, 2013).

THE SKIN PROVIDES A PROVING GROUND FOR hiPSC RESEARCH

With its easy accessibility, the skin is an attractive repository of materials for iPSC generation (Uitto, 2011). Cellular components of the skin, such as KCs, dermal cells, and melanocytes, have been successfully reprogrammed into hiPSCs (Takahashi *et al.*, 2007; Aasen *et al.*, 2008; Carey *et al.*, 2009; Utikal *et al.*, 2009; Figure 1). Interestingly, the advantage of using skin-derived cells for iPSC generation is not limited to their easy collectability. When transduced with four Yamanaka factors, human foreskin and hair follicle KCs far more efficiently gave rise to iPSCs compared with fibroblasts (Aasen *et al.*, 2008). Follicular dermal papilla cells and melanocytes were shown to upregulate SOX2 (Utikal *et al.*, 2009; Tsai *et al.*, 2011). Taking advantage of intrinsic upregulation of Klf4 as well as Sox2, murine dermal papilla cells were successfully dedifferentiated into iPSCs with Oct4 alone (Tsai *et al.*, 2011). Although, four factors were currently needed to reprogram human dermal papilla cells (Higgins *et al.*, 2012), reprogramming with fewer factors are theoretically possible. These suggest that constantly self-renewing skin contains stem cell populations of respective cell lineages, which may be less laboriously reprogrammed into iPSCs.

Following adoption of methodologies developed during the establishment of inducing KCs from human ES cells, KCs with the ability to reconstitute epidermal structures were induced from hiPSCs following exposure to retinoic acid and BMP4 (Itoh *et al.*, 2011; Veraitch *et al.*, 2013; Figure 1). Human melanocytes were also induced when iPSCs were converted into embryoid bodies and then incubated with endothelin-3, stem cell factor and WNT3A (Ohta *et al.*, 2011; Figure 1). Although, the complexity of *bona fide* skin is not currently reproduced, essential components for pigmented three-dimensional skin equivalent are available from hiPSCs. For experimental evaluation of iPSC-based therapy for SCI, hiPSC-derived NS/PCs need to be transplanted in the spinal cord and their biological behavior is not easy to monitor once implanted (Okano *et al.*, 2013; Figure 2a). In contrast, grafted hiPSC-skin equivalents are easily observable for the assessment of safety concerns, including long-term stability and tumorigenicity (Figure 2b). Accordingly, the skin has clear advantages over other organs in hiPSC investigation.

STEPWISE APPROACH FOR THE ESTABLISHMENT OF hiPSC-BASED TREATMENT

In order to establish iPSC-based therapy for impaired tissue recovery, several key issues need to be addressed in a stepwise manner: (1) clarification of protocols to direct undifferentiated stem cells toward full differentiation, (2) delineation of optimal environmental host conditions for engraftment of iPSC-derived cell therapies, (3) assessment of therapeutic efficacy of iPSC-derived tissues, and (4) determination of stability and safety of iPSC-derived therapies *in vivo*, particularly in humans. Using animal models (mostly rodents) for SCI and NS/PCs derived from ES cells/iPSCs, our group demonstrated that: (1) the subacute phase of SCI enabled the most efficient transplanted cell integration to the injured host neurons, (2) grafted hiPSC-NS/PCs prompted motor functional recovery after SCI not only in mice (Nori *et al.*, 2011) but also in common marmosets (Kobayashi *et al.*, 2012) without any sign of tumor formation, and (3) iPSC-NS/PCs that are able to give rise to neurons, astrocytes, and oligodendrocytes exert their therapeutic effects upon transplantation (Miura *et al.*, 2009; Tsuji *et al.*, 2010; Okano *et al.*, 2013; (Figure 2a)). We are

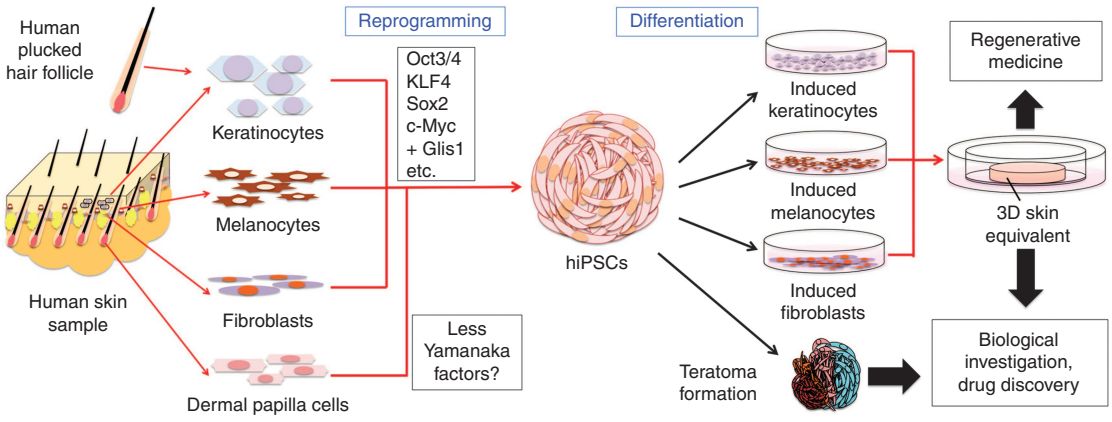

Figure 1. The skin provides a proving ground for human induced pluripotent stem cell (hiPSC) research. With its easy accessibility and high proliferative capacity, the skin represents an attractive repository of materials for hiPSC generation. Conversely, hiPCSs can be differentiated into keratinocytes, melanocytes, and dermal fibroblasts, which can be used for three-dimensional (3D) skin equivalent generation. hiPSCs also give rise to teratomas in which skin-like structures were reproduced.

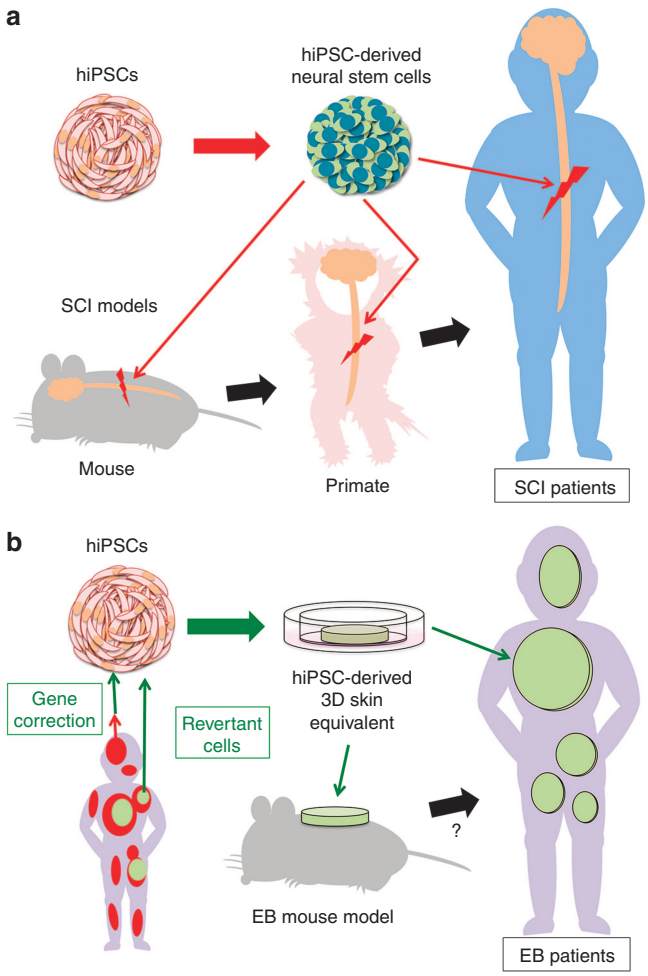

Figure 2. Approaches to establish human induced pluripotent stem cell (hiPSC)-based therapies for intractable disorders. (**a**) For the treatment of spinal cord injury (SCI), hiPSCs may be induced into neural stem/progenitor cells and transplanted to the injured spinal cord to support tissue regeneration. As hiPSC-derived tissue is permanently incorporated deep into the patient, the safety of transplanted tissue needs to be secured by the series of animal studies in a stepwise manner using mouse and non-human primate SCI models. (**b**) For the treatment of genodermatoses, especially epidermolysis bullosa (EB), gene correction is an indispensable step. hiPSCs may be generated from the cells obtained from the site showing revertant mosaicism or mutant cells were corrected for the gene mutation to generate hiPSCs. hiPSC-derived three-dimensional (3D) skin equivalent can be generated and grafted onto chronic wound of EB patients. As hiPSC-derived tissue is readily observable, the technique may be tried on human subjects, once long-term safety is secured with mouse EB models.

aware that not all those issues are directly applicable to iPSC-based treatment of skin injuries or defects. Some issues may be less important (for instance, tumorigenicity of grafted hiPSC-derived tissue is more easily monitored in the skin). Nevertheless, similar stepwise approach is required to achieve clinical application of bioengineered skin using hiPSCs.

iPSC-BASED TREATMENT FOR GENODERMATOSES

Use of hiPSCs for cell-based treatment of genodermatoses, in particular epidermolysis bullosa (EB), has been attracting great interest (Uitto, 2011; Figure 2b). A previous report suggested that adult KC stem cells might be exhausted in EB patients because of continuous need to repair wound (Mavilio *et al.*, 2006). As reprogramming rejuvenates adult somatic cells, using hiPSC-derived cells for EB treatment should be advantageous. When, KCs or fibroblast derived from recessive dystrophic EB patients were corrected for collagen VII gene mutation and subsequently reprogrammed into iPSCs, recessive dystrophic EB-hiPSC demonstrated the ability to differentiate into skin-like structure (Tolar *et al.*, 2011), suggesting that EB-hiPSC may be used for treating chronic wound in EB. More fascinating strategy is to take advantage of revertant mosaicism, in which a second spontaneous mutation in mutant cells cancels the primary mutation to reverse the phenotype to normal (Almaani *et al.*, 2010; Pasmooij *et al.*, 2010; Figure 2b). Generation of hiPSCs from revertant cells should enable patient-specific cell-based therapy for recessive dystrophic EB (Uitto, 2011). For small wound, skin equivalent generated from hiPSC-derived KCs and fibroblasts may be grafted (Figure 2b). Revertant cell-derived hiPSCs cells may also be induced into mesenchymal stem cells and infused into the circulation to be recruited into the wound site to differentiate into KCs or fibroblast to produce normal collagen VII (Chino *et al.*, 2008; Fujita *et al.*, 2010).

RECAPITULATION OF PATIENT PATHOPHYSIOLOGY USING iPSCs

hiPSCs also enable the reproduction of human disease pathophysiology. The familial form of Parkinson's disease, PARK2, is caused by mutation in the *parkin* gene, however, *parkin*-knockout mice do not fully recapitulate the pathophysiology of human PARK2. Interestingly, when hiPSCs were generated from PARK2 patients and differentiated into neurons, they exhibited mitochondrial dysfunction and α-synuclein accumulation observed in PARK2 patient tissue (Imaizumi *et al.*, 2012). KCs isolated from patient have been used to model the disease in organotypic cultures (König and Bruckner-Tuderman, 1994). KCs differentiated from patient-derived hiPSC should enable reproduction of patient pathophysiology in living skin. Indeed, three-dimensional skin equivalent generated with recessive dystrophic EB-hiPSC lacked collagen VII, therefore mimicking patient's skin (Itoh *et al.*, 2011). With their pluripotentcy, hiPSCs may be extremely useful for the investigation of pathomechanism of multi-organ diseases affecting skin, such as pesudoxanthoma elasticum or Ehlers–Danlos syndrome. Thus, diseases models utilizing hiPSCs for such disorders should provide

powerful tools for better understanding of their pathophysiology and pharmacological target discovery.

POSSIBLE STRATEGIES FOR SKIN APPENDAGE REGENERATION USING hiPSCs

Morphogenesis of skin appendages depends on intensive and orchestrated epithelial–mesenchymal interactions. Previous study demonstrated embryonic or neonatal human KCs more efficiently generated hair follicle structure than adult KCs, when combined with hair inductive dermal cells in *in vivo* hair reconstitution, suggesting that innate state KC could better respond to inductive signals (Ohyama and Veraitch, 2013). Technically, it is difficult to recondition adult KCs back into an embryonic/neonatal state. However, hiPSC-derived KC lineage cells with high receptivity to dermal signals may be obtained during the differentiation from hiPSCs to terminally differentiated KCs (Ohyama and Veraitch, 2013). To support this hypothesis, hiPSC-derived ectodermal precursors (hiPSC-EPCs) expressing keratin 18 and partially keratin 14 (thus, less committed to KC lineage) more intensely crosstalk with human dermal papilla cells than adult KCs to express higher levels of hair-related genes (Veraitch *et al.*, 2013). Importantly, hiPSC-EPCs, but not adult KCs, contributed to hair follicle structures in hair reconstitution experiments (Veraitch *et al.*, 2013). These findings implied the advantage of using hiPSC-derived cells to provoke epithelial–mesenchymal interactions sufficient for tissue bioengineering.

FUTURE OF IPSC-BASED MEDICINE IN DERMATOLOGY

As described above, hiPSCs holds great promise in dermatology. However, hiPCSs should not be considered as ''omnipotent'' in every application. Of note, safety issues need to be completely cleared for their use in clinics. Generation of clinical-grade, integration-free, xeno-free, and more completely reprogrammed hiPSCs is definitely necessary (Nakamura and Okano, 2013; Okano *et al.*, 2013). Some essential techniques for skin regeneration and transplantation have been already established. Considered combination of conventional approaches with iPSC technology should accelerate the advances in regenerative medicine and facilitate new discoveries in the field of skin biology.

CONFLICT OF INTEREST

HO is a member of the scientific advisory boards of San Bio, Eisai, and Daiichi Sankyo. MO states no conflict of interest.

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REFERENCES

Aasen T, Raya A, Barrero MJ *et al.* (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 26:1276–84

Almaani N, Nagy N, Liu L *et al.* (2010) Revertant mosaicism in recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 130:1937–40

Amabile G, Welner RS, Nombela-Arrieta C *et al.* (2013) *In vivo* generation of transplantable human hematopoietic cells from induced pluripotent stem cells. *Blood* 121:1255–64

Barrandon Y, Green H (1987) Three clonal types of keratinocyte with different capacities for multiplication. *Proc Natl Acad Sci USA* 84: 2302–6

Blanpain C, Fuchs E (2009) Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol* 10:207–17

Carey BW, Markoulaki S, Hanna J *et al.* (2009) Reprogramming of murine and human somatic cells using a single polycistronic vector. *Proc Natl Acad Sci USA* 106:157–62

Chino T, Tamai K, Yamazaki T *et al.* (2008) Bone marrow cell transfer into fetal circulation can ameliorate genetic skin diseases by providing fibroblasts to the skin and inducing immune tolerance. *Am J Pathol* 173:803–14

Cotsarelis G (2006) Epithelial stem cells: a folliculocentric view. *J Invest Dermatol* 126: 1459–68

Fujita Y, Abe R, Inokuma D *et al.* (2010) Bone marrow transplantation restores epidermal basement membrane protein expression and rescues epidermolysis bullosa model mice. *Proc Natl Acad Sci USA* 107:14345–50

Green H, Kehinde O, Thomas J (1979) Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. *Proc Natl Acad Sci USA* 76:5665–8

Harding J, Roberts RM, Mirochnitchenko O (2013) Large animal models for stem cell therapy. *Stem Cell Res Ther* 4:23

Higgins CA, Itoh M, Inoue K *et al.* (2012) Reprogramming of human hair follicle dermal papilla cells into induced pluripotent stem cells. *J Invest Dermatol* 132:1725–7

Imaizumi Y, Okada Y, Akamatsu W *et al.* (2012) Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. *Mol Brain* 5:35

Itoh M, Kiuru M, Cairo MS *et al.* (2011) Generation of keratinocytes from normal and recessive dystrophic epidermolysis bullosa-induced pluripotent stem cells. *Proc Natl Acad Sci USA* 108:8797–802

Jahoda CA, Whitehouse J, Reynolds AJ *et al.* (2003) Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. *Exp Dermatol* 12:849–59

Jensen KB, Watt FM (2006) Single-cell expression profiling of human epidermal stem and transit-amplifying cells: Lrig1 is a regulator of stem cell quiescence. *Proc Natl Acad Sci USA* 103:11958–63

Kattman SJ, Witty AD, Gagliardi M *et al.* (2011) Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. *Cell Stem Cell* 8:228–40

Kobayashi Y, Okada Y, Itakura G *et al.* (2012) Pre-evaluated safe human iPSC-derived neural stem cells promote functional recovery after spinal cord injury in common marmoset without tumorigenicity. *PLoS One* 7:e52787

König A, Bruckner-Tuderman L (1994) Transforming growth factor-beta promotes deposition of collagen VII in a modified organotypic skin model. *Lab Invest* 70:203–9

Kuroda Y, Kitada M, Wakao S *et al.* (2010) Unique multipotent cells in adult human mesenchymal cell populations. *Proc Natl Acad Sci USA* 107:8639–43

Li A, Simmons PJ, Kaur P (1998) Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. *Proc Natl Acad Sci USA* 95:3902–7

Mavilio F, Pellegrini G, Ferrari S *et al.* (2006) Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. *Nat Med* 12:1397–402

Miura K, Okada Y, Aoi T *et al.* (2009) Variation in the safety of induced pluripotent stem cell lines. *Nat Biotechnol* 27:743–5

Nakamura M, Okano H (2013) Cell transplantation therapies for spinal cord injury focusing on induced pluripotent stem cells. *Cell Res* 23:70–80

Nori S, Okada Y, Yasuda A *et al.* (2011) Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice. *Proc Natl Acad Sci USA* 108:16825–30

Ohta S, Imaizumi Y, Okada Y *et al.* (2011) Generation of human melanocytes from induced pluripotent stem cells. *PLoS One* 6:e16182

Ohyama M, Terunuma A, Tock CL *et al.* (2006) Characterization and isolation of stem cell-enriched human hair follicle bulge cells. *J Clin Invest* 116:249–60

Ohyama M, Veraitch O (2013) Strategies to enhance epithelial-mesenchymal interactions for human hair follicle bioengineering. *J Dermatol Sci* 70:78–87

Okano H, Nakamura M, Yoshida K *et al.* (2013) Steps toward safe cell therapy using induced pluripotent stem cells. *Circ Res* 112:523–33

Pasmooij AM, Garcia M, Escamez MJ *et al.* (2010) Revertant mosaicism due to a second-site mutation in COL7A1 in a patient with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 130:2407–11

Pellegrini G, Ranno R, Stracuzzi G *et al.* (1999) The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. *Transplantation* 68:868–79

Takahashi K, Tanabe K, Ohnuki M *et al.* (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861–72

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–76

Tolar J, Xia L, Riddle MJ *et al.* (2011) Induced pluripotent stem cells from individuals with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 131:848–56

Toma JG, McKenzie IA, Bagli D *et al.* (2005) Isolation and characterization of multipotent skin-derived precursors from human skin. *Stem Cells* 23:727–37

Tsai SY, Bouwman BA, Ang YS *et al.* (2011) Single transcription factor reprogramming of hair follicle dermal papilla cells to induced pluripotent stem cells. *Stem Cells* 29:964–71

Tsuji O, Miura K, Okada Y *et al.* (2010) Therapeutic effect of the appropriatelly evaluated 'safe' iPS cells for spinal cord injury. *Proc Natl Acad Sci USA* 107:12704–9

Uitto J (2011) Regenerative medicine for skin diseases: iPS cells to the rescue. *J Invest Dermatol* 131:812–4

Utikal J, Maherali N, Kulalert W *et al.* (2009) Sox2 is dispensable for the reprogramming of melanocytes and melanoma cells into induced pluripotent stem cells. *J Cell Sci* 122:3502–10

Veraitch O, Kobayashi T, Imaizumi Y *et al.* (2013) Human induced pluripotent stem cell-derived ectodermal precursor cells contribute to hair follicle morphogenesis in vivo. *J Invest Dermatol* 133:1479–88