Regenerative Medicine for Skin Diseases: iPS Cells to the Rescue

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Induced pluripotent stem (iPS) cells reprogrammed from somatic cells have the potential to differentiate, under appropriate conditions, to any cell type. Recent studies, including two papers in this issue (Bilousova et al., 2011; Tolar et al., 2011), have demonstrated that iPS cells can differentiate into keratinocytes. Thus, iPS cells may provide a novel approach to applying regenerative medicine to cutaneous diseases such as epidermolysis bullosa.


At the 2010 Annual Meeting of the Society for Investigative Dermatology in Atlanta, Georgia, among the hot topics were several abstracts reporting that induced pluripotent stem (iPS) cells can be differentiated into keratinocytes, suggesting that such cells can be applied to regenerative medicine in dermatology. Now, several months later, the full papers on these presentations are appearing in the literature. In this issue, two articles (Bilousova et al., 2011; Tolar et al., 2011) report on differentiation of mouse and human iPS cells into keratinocytes. A complementary study (Itoh and Christiano, 2010) is being prepared for publication (Itoh et al., personal communication). The translational focus of these papers centers on epidermolysis bullosa (EB), a group of heritable blistering diseases in which, as a result of mutations in as many as 14 different genes expressed in the epidermis and the cutaneous basement membrane zone, the skin and mucous membranes are extremely fragile, leading to blistering and erosions with considerable morbidity and mortality. No effective or specific treatment is currently available for EB, but significant progress has been made recently toward the development of DNA-, protein-, and cell-based therapies (Uitto et al., 2010). Generation of functional skin from iPS cells may now provide an innovative approach to enhancing the regeneration and repair of damaged tissues in these patients.

**Generation and differentiation of iPS cells**

The development and use of iPS cells comprise a rapidly evolving field that emerged in 2006 with the demonstration that somatic cells, such as skin fibroblasts (Takahashi and Yamanaka, 2006), epidermal keratinocytes (Asen et al., 2008), and hair follicle outer root sheath cells (Asen and Belmonte, 2010) from adults can be reprogrammed to an embryonic stem (ES) cell-like state by addition of a few selected transcription factors (c-MYC, SOX2, OCT4, and KLF4) (Figure 1). Culturing and characterization of such cells then allow isolation of pluripotent cells with features of ES cells and the potential to differentiate into any cell type in the body. Several characteristics indicate the successful generation of iPS cells (Table 1), including a morphology similar to that of human ES cells (i.e., small size, prominent nuclei with a large nucleus-to-cytoplasm ratio, and tight and flat colonies with clear-cut, round edges) and a normal karyotype. Expression of several stem cell markers must be demonstrated by immunostaining or by reverse transcription–PCR—particularly expression of the NANO gene, which is not used for induction of iPS cells. These reprogramming factors are thought to activate a network of transcriptional factors that in turn induce epigenetic changes, including demethylation of OCT4 and NANO promoter sequences. The pluripotent capacity of these cells is then demonstrated by teratoma formation in vivo upon injection of iPS cells into immunocompromised mice, and confirming the contribution of iPS cells to cell lineages of endodermal, mesodermal, and ectodermal origin using germ layer-specific markers.

Once newly generated iPS cells have been characterized and their pluripotent capacity has been verified, they can theoretically be differentiated into any one of several lineages in vitro using culture conditions that are directed toward each lineage. This general scheme has been shown to be successful in generating specialized, well-differentiated cells, such as cardiomyocytes, neural cells, osteoblasts, blood progenitor cells, and insulin-producing cells. The three papers cited above are the first to demonstrate differentiation of iPS cells to keratinocytes, albeit using different, complementary methods and approaches.

**Induced pluripotent cells can differentiate into keratinocytes for the regeneration of skin.**

**From iPS cells to skin**

The study by Bilousova et al. (2011) demonstrates directed differentiation of mouse iPS cells in culture into a multipotent keratinocyte lineage capable of forming a fully differentiated epidermis, hair follicles, and sebaceous glands and in a reconstitutive in vivo environment. The key in differentiating iPS cells toward the ectodermal lineage is the sequential application of retinoic acid (RA) and bone morphogenetic protein 4 (BMP4), which resulted in keratin 14-positive epidermal stem cells. This stem cell population could be enriched by selective attachment to type IV collagen-coated surfaces, onto which the fully differentiated progeny does not attach.
The study by Itoh et al. (personal communication) also reports successful generation of iPS cells, not only from normal human fibroblasts but also from fibroblasts isolated from the skin of patients with recessive dystrophic EB (RDEB). These investigators employed similar culture conditions using RA and BMP4, which allowed expedient differentiation of these iPS cells into keratinocytes. Importantly, these cells were used to generate three-dimensional skin equivalents, suggesting that they were fully functional.

It is noteworthy that both of these studies drew on a foundation of knowledge in ES cell biology that has emerged in recent years. For example, both studies borrowed a page from earlier work attesting to the utility of RA and BMP4 in differentiating ES cells into keratinocytes and subsequent reprogramming of keratinocytes into a neural fate (Aberdam, 2004; Grinnell and Bickenbach, 2007). In addition, the succession of markers that are activated as ES cells differentiate into keratinocytes provides a road map for monitoring the efficacy of directed differentiation of iPS cells into keratinocytes (Green et al., 2003).

The study by Tolar et al. (2011) takes a different approach. Instead of directed differentiation into keratinocytes in culture followed by skin-reconstitution assessment, these investigators used direct injection and teratoma formation to allow spontaneous differentiation of iPS cells into skin-like structures expressing keratinocyte markers. As a starting point, they used human fibroblasts or keratinocytes from normal individuals as well as from patients with RDEB, and they performed gene correction of the latter cells with a vector expressing type VII collagen. The differentiation of RDEB cells into corresponding iPS cells was similar to that observed for wild-type iPS cells, including the ability to differentiate spontaneously into structures resembling skin. Interestingly, these investigators also observed differentiation of these cells into hematopoietic lineages, which suggests that such cells could be used to generate autologous hematopoietic cells for grafting. Collectively, these studies suggest that iPS cells can be differentiated into keratinocytes and skin-like structures, with the potential of treating EB.

Revertant mosaicism: a unique opportunity for cell-based therapy

An intriguing possibility related to the use of iPS cells for regenerative medicine in dermatology centers on the potential of combining iPS cell technology with revertant mosaicism, which clinically manifests as patches of normal skin in patients with heritable skin diseases such as EB (Almaani et al., 2010; Pasmooj et al., 2010). These areas of skin reflect the presence of a second mutation in the clonal population of cells—as a result of back mutations, intragenic crossovers, or gene conversion—that negates the deleterious effects of the primary mutation and reverses the phenotype; this phenomenon has been dubbed “natural gene therapy” (Lai-Cheong et al., 2011). Thus, generation of iPS cells from spontaneously revertant skin would provide an essentially unlimited number of patient-specific cells for grafting in diseases such as RDEB. Collectively, these studies attest to the potential of iPS cells for patient-specific stem cell therapy for skin diseases.

iPS cells: prospects and promises

The advantages of utilizing iPS cells for regenerative medicine over the ES cells are several. For example, the use of iPS cells obviates the ethical/political issues that have surrounded ES cells, especially in the United States. iPS cells can be autologous and patient specific, eliminating the issues relating to immune-based rejection of the grafts. Also, the generation of iPS cells can theoretically be scaled up, essentially providing an unlimited source of cells for medical application. So, when will iPS cell therapy be available for patients? Stem cell researchers agree that utility of iPS cells for medical applications will be a reality eventually but that their use is only in the early days of development (Vogel, 2010). Among the several areas of uncertainty is the possibility that the transgenes encoding reprogramming factors, which are ordinarily inserted into target cells via retroviral vectors, may cause carcinogenesis and that inactivation of the viral genes will be incomplete, possibly leading to tumor formation. Okita et al. (2008), seeking to circumvent the use of viral genes for transfection, found that introduction of the corresponding transcription factors in the form of proteins delivered to the cells allows induction of iPS cells without viral vectors.

Even more intriguing is the recent demonstration that a simple, nonintegrating strategy of administering synthetic mRNAs for the transcription factors can result in rapid and efficient reprogramming of cells to pluripotency.

Table 1. Criteria for successful generation of induced pluripotent stem cells

| Characteristic morphology and normal karyotype |
| Immunostaining and increased gene expression of stem cell markers (OCT4, SOX2, NANOG) |
| Silencing of the viral transgenes |
| Demethylation of stem cell gene promoters (NANOG) |
| Teratoma formation in vivo and contribution to all three germ layers |

Figure 1. Schematic steps of reprogramming somatic cells, such as fibroblasts, to induced pluripotent stem (iPS) cells and their differentiation into epidermal keratinocytes capable of forming skin-like structures. The reprogramming process is initiated by the introduction of transcription factors (c-MYC, SOX2, OCT4, and KLF4) into the somatic cells by transduction of expression vectors, synthetic mRNA, or recombinant protein. The iPS cells have characteristic features that allow their identification and enrichment (see Table 1). The iPS cells can then be differentiated into keratinocytes under specific culture conditions, e.g., medium supplemented with retinoic acid (RA) and bone morphogenic protein-4 (BMP-4). BMZ, basement membrane zone.

www.jidonline.org 813
Clinical Implications

- Generation of iPS cells provides an essentially unlimited source of cells with the potential to differentiate into any cell type in the body.
- iPS cells have been shown to differentiate into keratinocytes with the capacity to form skin-like structures.
- iPS cells may provide a personalized, patient-specific approach to treat skin diseases such as EB.

(Warren et al., 2010). Because the exog- enously administered RNA molecules are rapidly degraded, the DNA back- bone of the reprogrammed cells remains intact and is indistinguishable from that of the original wild-type cells. Finally, the use of iPS cells to treat heritable skin diseases will require transduction of the missing or nonfunctional gene to the cells (such as type VII collagen into iPS cells generated from patients with RDEB) for patients who do not display revertant mosaicism. Here again, the question of carcinogenesis, even if a hypothetical possibility, has been raised when retroviral vectors have been used to insert the transgene. Along these lines, gene- correction strategies, such as zinc-finger nuclease technology, have emerged as viable alternatives for the repair of mutations in iPS cells (Urnov et al., 2010).

It is clear at this point that iPS cells can provide a useful model for studying the mechanisms of diseases, and these cell systems can be used for large-scale screens in the development of candidate drugs. Novel technologies relating to iPS cells are rapidly being developed (Csete, 2010; Warren et al., 2010), and the three papers highlighted above mark a significant milestone in skin biology because they demonstrate differentiation of both human and mouse iPS cells into keratino- cytes. Refinements in the generation of such cells may provide treatment options for heritable skin diseases such as RDEB sooner than we realize.

CONFLICT OF INTEREST
The author states no conflict of interest.

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Pulling RANK: Are Some Melanoma Cells More Malignant than Others?

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Cellular heterogeneity is a frequently noted feature of melanoma. As reported in this issue, Kupas et al. identified heterogeneous expression of receptor activator of nuclear factor-κB (RANK) in melanoma cells in tumors and peripheral blood from patients. Increased expression of RANK was associated with the presence of metastatic disease and increased tumorigenicity in melanoma cells, raising the possibility that RANK signaling contributes to melanoma progression.

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Melanoma kills patients primarily because of its propensity to metastasize distantly via the bloodstream. This indicates that at least some melanoma cells that enter the circulation after dislodging from tumors must not only harbor intrinsic malignant

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