Editorial

Induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa

Stavros Spiliopoulos[1], Nikolaos Davanos[2]

[1]Department of Interventional Radiology, Patras University Hospital, Rio, Greece; [2]Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Patras, Rio, Greece

Correspondence to: Dr. Stavros Spiliopoulos, MD, PhD, EBIR. Department of Interventional Radiology, Patras University Hospital, Rio, Greece. Email: stavspiliop@upatras.gr.

Submitted Sep 03, 2015. Accepted for publication Sep 09, 2015.
doi: 10.3978/j.issn.2305-5839.2015.09.42
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.09.42

Epidermolysis bullosa (EB) is a group of genetically inherited skin disorders affecting nearly 1 to 3 of every 100,000 live births and almost 500,000 individuals worldwide (1). Autosomal recessive dystrophic EB (RDEB) is an aggressive EB subtype (approximately 25% of EB cases), caused by *COL7A1* gene mutation encoding type VII collagen and resulting in binding defect of epidermis to dermal tissue, excessive skin and mucosal fragility, blistering and scarring. Lesions occur below the basement membrane zone in the upper part of the dermis. Until today no definite therapy is available and patients suffering from RDEB are managed conservatively avoiding skin damage and aiming in improve quality of life and reducing the risk of developing complications, such as infection and malnutrition, or undergo surgical or minimal invasive palliative procedures (2,3). Current research strategies include protein, gene and molecular therapies (4-6). In their article "Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa" Sebastiano *et al*. propose an innovating cell therapy for RDEB treatment, by developing a state of the art protocol of genetically repaired induced pluripotent stem cells (iPSCs) as to generate sheets of normal skin tissue to treat affected skin areas (7). Moreover, as numerous stem cells are needed in order to cover the affected surface area, authors outline the necessity for creating personalized iPSCs banks as to provide a constant long-term iPSCs source.

Generally, human iPSCs can be generated by reprogramming differentiated somatic cells into pluripotent embryonic stem cells (ESCs) capable of differentiating into ectoderm, mesoderm or endoderm cells. Reprogramming involves the introduction of a known set of genes into the somatic cells, using integrating viral and non-integrating non-viral methods. Following successful reprogramming, somatic cells will express genes and surface proteins similar to ESCs *in vitro* and will be able to differentiate into any of the three embryonic germ layers (8).

Patient derived differentiated somatic cells can be reprogrammed to iPSCs for a number of genetic diseases (9). Patient specific iPSCs can then be corrected *in vitro* to repair the disease causing mutation. The wild type sequence of the defective gene can be delivered by means of genome editing. This is typically achieved by high frequency homologous recombination (HR) using recombinant adeno-associated viral (AAV) targeting vectors or non-homologous end joining (NHEJ) and low frequency HR using site directed endonuclease double stranded DNA brakes. Following successful genome editing, the iPSCs can be differentiated into phenotypically corrected, cancer mutation-free selected somatic cells *in vitro*, which can be exploited to regenerate autologous tissue.

The present study describes an *in vitro* methodology for (I) reprogramming fibroblasts and keratinocytes from RDEB patients into iPSCs; (II) correcting patient derived iPSCs at the COL7A1 locus and (III) differentiating corrected iPSCs into collagen type VII producing keratinocytes capable of regenerating skin tissue *in vivo*.

For the production of clinical grade iPSCs the authors used reagents qualified per US Food and Drug Administration (FDA) standards under good manufacturing procedures (GMP). Integrating viral delivery systems for both reprogramming and genome editing methodologies

were used in order to achieve better control over tracking genomic modifications throughout the sequential process of patient derived iPSC production and correction.

Reprogramming was performed by transduction of patient derived fibroblasts and keratinocytes with a recombinant lentivirus containing a known set of transcription factors. Following successful transduction, iPSC colonies were morphologically assessed and expanded, stained for pluripotency markers and karyotyped. Proviral integration was confirmed with Southern blotting. The ability to differentiate into cells of each of the three embryonic germ layers was confirmed by teratoma formation. Following reprogramming, the AAV-DJ variant was used to deliver the correct CO7A1 sequence in the patient derived iPSCs. Infected clones were selected and assayed for HR and correct targeting using Southern blotting and Sanger sequencing. The selection marker and the integrated reprogramming factors were then looped-out by transient Cre recombinase expression. Looping-out was confirmed by PCR and Southern blot analysis of patient derived iPSCs. Normal karyotype was confirmed by cytogenetic analysis. No random integrations of AAV were confirmed by Southern blotting. Targeting and correction efficiencies of the AAV system were comparable to conventional and site directed endonuclease targeting methodologies.

Genetic variation of different iPSC production stages was assessed by sequencing patient derived somatic cells, patient derived iPSCs and corrected looped-out iPSCs using a whole-genome sequencing platform (complete genomics) and the Ingenuity Variant Analysis software. Although high heterogeneity was evident in all cell lines, there was no indication of a driving mechanism such as source cell inheritance or introduction by reprogramming (10).

A targeted re-sequencing methodology was then employed to test corrected looped-out iPSCs for known mutations in squamous cell carcinoma (SCC) predisposition genes. This allowed the selection of iPSCs with the fewer SCC-associated mutations for downstream applications.

For tissue manufacturing purposes, the authors developed a protocol to differentiate corrected looped-out SSC mutation-free iPSCs into pure cultures of functional keratinocytes that could be grown into epithelial sheets to restore adhesion in the skin. The results confirmed that embryoid body formation and iPSC growth on feeders before differentiation improves efficiency and reduces heterogeneity of the keratinocyte culture (11). Differentiation into keratinocytes was morphologically tested and the homogeneity of the final culture was demonstrated by fluorescence-activated cell sorting (FACS) analysis. A microarray gene expression assay was employed to confirm the resemblance of corrected iPSC derived keratinocytes, patient derived keratinocytes and neonatal human keratinocytes (NHK). Western blot analysis was employed to demonstrate the production of type VII collagen in the corrected iPSC derived patient keratinocytes. Functionality was assessed by an *in vitro* skin reconstitution assay and *in vivo* by performing xenografts onto immunocompromised mice, a validated preclinical model for human epithelial sheet formation (12).

Overall, this is a well-designed and complete workflow describing the production of clinical grade COL7A1 mutation corrected iPSCs from RDEB patient derived fibroblasts and keratinocytes using viral excisable vectors. All necessary assays were employed for the characterization and qualification of iPSCs throughout the manufacturing procedure. Under specific conditions these cells can then be differentiated into functional corrected keratinocytes capable of depositing type VII collagen. The present study underlines the necessity of detection and monitoring for genetic variations in iPSC manufacturing using next generation sequencing approaches, in order to avoid using problematic cell lines for downstream applications. Limitations of this protocol include short-term (3-week) duration of genetically manufactured skin tissue and the necessity of further investigation in a disease model. Moreover, more accurate and rigorous standards of iPSC clones characterization are required as to develop safe and practical clinical protocols. Nonetheless, authors demonstrated that manufacturing normal skin tissue using genetically repaired autologous iPSCs is feasible, leading the way to the future application of personalized regenerative medicine for the treatment of RDEB patients.

Acknowledgements

None.

Footnote

Provenance: This is a Guest Editorial commissioned by the Section Editor Ning Ding, PhD (Department of Respiratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Horn HM, Tidman MJ. The clinical spectrum of dystrophic epidermolysis bullosa. Br J Dermatol 2002;146:267-74.
2. Spiliopoulos S, Sabharwal T, Krokidis M, et al. Fluoroscopically guided dilation of esophageal strictures in patients with dystrophic epidermolysis bullosa: long-term results. AJR Am J Roentgenol 2012;199:208-12.
3. Shinkuma S. Dystrophic epidermolysis bullosa: a review. Clin Cosmet Investig Dermatol 2015;8:275-84.
4. Woodley DT, Keene DR, Atha T, et al. Injection of recombinant human type VII collagen restores collagen function in dystrophic epidermolysis bullosa. Nat Med 2004;10:693-5.
5. Mavilio F, Pellegrini G, Ferrari S, et al. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nat Med 2006;12:1397-402.
6. Hou Y, Guey LT, Wu T, et al. Intravenously administered recombinant human type VII collagen derived from chinese hamster ovary cells reverses the disease phenotype in recessive dystrophic epidermolysis bullosa mice. J Invest Dermatol 2015;135:3060-7.
7. Sebastiano V, Zhen HH, Haddad B, et al. Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. Sci Transl Med 2014;6:264ra163.
8. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-72.
9. Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. Cell 2008;134:877-86.
10. Liang G, Zhang Y. Genetic and epigenetic variations in iPSCs: potential causes and implications for application. Cell Stem Cell 2013;13:149-59.
11. Metallo CM, Ji L, de Pablo JJ, et al. Retinoic acid and bone morphogenetic protein signaling synergize to efficiently direct epithelial differentiation of human embryonic stem cells. Stem Cells 2008;26:372-80.
12. Antonchuk J. Formation of embryoid bodies from human pluripotent stem cells using AggreWell™ plates. Methods Mol Biol 2013;946:523-33.

Cite this article as: Spiliopoulos S, Davanos N. Induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. Ann Transl Med 2015;3(22):349. doi: 10.3978/j.issn.2305-5839.2015.09.42

