Title
Melanocytes derived from transgene-free human induced pluripotent stem cells.

Permalink
https://escholarship.org/uc/item/4zp3c8b2

Journal
The Journal of investigative dermatology, 133(8)

ISSN
0022-202X

Authors
Jones, Jennifer C
Sabatini, Karen
Liao, Xiaoyan
et al.

Publication Date
2013-08-01

DOI
10.1038/jid.2013.139

Peer reviewed
Melanocytes Derived from Transgene-Free Human Induced Pluripotent Stem Cells

Journal of Investigative Dermatology (2013) 133, 2104–2108; doi:10.1038/jid.2013.139; published online 9 May 2013

TO THE EDITOR

Defects in melanocytes have been implicated in the etiology of a variety of human skin diseases and disorders (Lin and Fisher, 2007; Fistarol and Itin, 2010; Rees, 2011). There is long-standing interest in studying the development and dysfunction of human melanocytes, but there has not been a reliable and accessible system to study early events in human melanocyte differentiation. An in vitro system that reliably and efficiently produces normal human melanocytes from embryonic stage cells would allow us to better dissect the physiological and pathological development of melanocytes. Recent advances in stem cell biology have led to the establishment of human induced pluripotent stem cell (hiPSC) techniques that enable researchers to reprogram somatic cells to the pluripotent state (Takahashi et al., 2007). Differentiation of human and mouse pluripotent stem cells (PSCs) toward the melanocyte lineage has been reported (Yamane et al., 1999; Pla et al., 2005; Fang et al., 2006; Nissan et al., 2011; Ohta et al., 2011; Yang et al., 2011), but existing protocols have shortcomings that may limit their research and clinical applications. For example, the use of embryonic stem cells could lead to allogeneic immunoincompatibility of differentiated melanocytes and transplant recipients. In addition, the use of hiPSCs generated by integrative reprogramming strategies raises concerns about reactivation of retained transgenes, some of which are oncogenes. In addition, the current methods for melanocyte differentiation from hiPSCs require optimization in order to reproducibly generate high-purity melanocytes from multiple hiPSC lines.

We have established a strategy to produce human melanocytes in vitro for use as a platform for pigment cell research and the development of cell-based therapies. We first derived transgene-free hiPSCs from two distinct types of skin cells: human primary melanocytes (HMs) and human dermal fibroblasts.

---

**Abbreviations:** hiPSC, human induced pluripotent stem cell; HM, human primary melanocyte; α-MSH, α-melanocyte-stimulating hormone; MITF, microphthalmia-associated transcription factor; PSC, pluripotent stem cell; SNP, single-nucleotide polymorphism

**Accepted article preview online 20 March 2013; published online 9 May 2013**
Figure 1. For caption see page 2106.
Figure 1. Generation and differentiation of transgene-free human induced pluripotent stem cell (hiPSCs). (a) HMi-506 cells generated from human primary melanocyte (HM) cells using a Sendai virus–based reprogramming system were cocultured with mouse embryonic fibroblast feeder cells (upper panel) and in feeder-free conditions (lower panel). (b) Immunofluorescence staining showed that biomarkers of pluripotency, Tra-1-81, NANOG, OCT4/POU5F1, and UEA-I lectin, were strongly positive in HMi-506 cells but absent in their differentiated derivatives (Mel Diff) and HM cells. (c) Embryoid bodies from HMi-506 cells contained cells from all three germ layers. NG2-positive cells: ectoderm; smooth muscle actin (SMA)–positive cells: mesoderm; SOX17-positive cells: endoderm. (d) The HMi-506_Mel Diff cells displayed pigmentation and morphology typical of HM cells. The black arrow indicates a pigmented cell pellet of HMi-506_Mel Diff cells, whereas the white arrow indicates undifferentiated HMi-506 cells. (e) Immunofluorescence staining showed that microphthalmia-associated transcription factor (MITF) was expressed in HM cells and HMi-506_Mel Diff cells and absent in HMi-506 cells. DAPI, 4’,6-diamidino-2-phenylindole. Scale bars = 100 μm.

Figure 2. Continued on following page.
Mediated by Sendai virus–based vectors

A nonintegrative reprogramming approach (HDF51) (Figure 1a and Supplementary Figure S1a online). We used a (HDF51) (Figure 1a and Supplementary Figure S1a online). We used a

Many processes, we tested the genomic stability of the reprogramming and differentiation

cells showing these transcripts were preferentially expressed in human primary melanocyte (HM) cells and HMi-506_Mel Diff cells. Brown arrows, HM samples; turquoise arrows, undifferentiated HMi-506 samples; orange arrows, HMi-506_Mel Diff samples.抄袭 scripts were detected by western blotting. (c) Melanin production was increased in a dose-dependent manner in the differentiated derivatives treated with α-melanocyte-stimulating hormone (α-MSH) for 48 hours. Columns indicate mean of three independent experiments and bars indicate SD. (d) Protein expression of TYR and MITF-H4 in cells subjected to α-MSH treatment for 24 hours detected by western blotting. (e) Unsupervised hierarchical clustering of gene expression profiles from 22 human pluripotent stem cell (hiPSC) samples and 17 samples of human nonpluripotent cells. Arrows were colored as in a. (f) Copy number variation (CNV) analysis was performed using single-nucleotide polymorphism (SNP) genotyping data, and indicated that no significant additional CNVs arise during reprogramming or differentiation.

In this study, we have demonstrated that genetically stable melanocytes can be efficiently differentiated from transgene-free hiPSCs generated from two different types of cutaneous cells. This differentiation protocol takes less time than previously reported melanocytic differentiation protocols, and we showed that it is equally effective for multiple independent hiPSC lines. We performed a thorough investigation of the differentiated cells, including genome-wide gene expression analysis and SNP genotyping in addition to functional assays. Our approach can serve as an unlimited source of custom human primary melanocytes using high-resolution single-nucleotide polymorphism (SNP) genotyping and copy number variation analysis. As shown in Figure 2f, the HMi-506_Mel Diff derivatives and parental cells showed highly similar genotyping profiles, showing that the cellular genome remained stable during reprogramming and differentiation.

Similar to human melanocytes in vivo, the differentiated derivatives in semiautologous skin reconstructions were located at the dermis–epidermis interface and interspersed with keratinocytes (Supplementary Figure S6a, S6b, S6c and S6d online), indicating their ability to integrate with the skin tissue of transplant recipients. Similar to the autologous dermal fibroblasts used for generating transgene-free hiPSCs, the differentiated derivatives stimulated limited proliferation of peripheral blood mononuclear cells that were isolated from the blood of the same individual in a mixed lymphocyte reaction assay (Supplementary Figure S6e online). These results attest to the clinical advantages of melanocytes differentiated from hiPSCs using the reprogramming and differentiation approaches described here.

In this study, we have demonstrated that genetically stable melanocytes can be efficiently differentiated from transgene-free hiPSCs generated from two different types of cutaneous cells. This differentiation protocol takes less time than previously reported melanocytic differentiation protocols, and we showed that it is equally effective for multiple independent hiPSC lines. We performed a thorough investigation of the differentiated cells, including genome-wide gene expression analysis and SNP genotyping in addition to functional assays. Our approach can serve as an unlimited source of custom human primary melanocytes using high-resolution single-nucleotide polymorphism (SNP) genotyping and copy number variation analysis. As shown in Figure 2f, the HMi-506_Mel Diff derivatives and parental cells showed highly similar genotyping profiles, showing that the cellular genome remained stable during reprogramming and differentiation.

In this study, we have demonstrated that genetically stable melanocytes can be efficiently differentiated from transgene-free hiPSCs generated from two different types of cutaneous cells. This differentiation protocol takes less time than previously reported melanocytic differentiation protocols, and we showed that it is equally effective for multiple independent hiPSC lines. We performed a thorough investigation of the differentiated cells, including genome-wide gene expression analysis and SNP genotyping in addition to functional assays. Our approach can serve as an unlimited source of custom human primary melanocytes using high-resolution single-nucleotide polymorphism (SNP) genotyping and copy number variation analysis. As shown in Figure 2f, the HMi-506_Mel Diff derivatives and parental cells showed highly similar genotyping profiles, showing that the cellular genome remained stable during reprogramming and differentiation.

Melanocytes Derived from Transgene-Free hiPSCs

JC Jones et al.

Melanocytes Derived from Transgene-Free hiPSCs

JC Jones et al.

Melanocytes Derived from Transgene-Free hiPSCs

JC Jones et al.

Melanocytes Derived from Transgene-Free hiPSCs

JC Jones et al.

Melanocytes Derived from Transgene-Free hiPSCs

JC Jones et al.

Melanocytes Derived from Transgene-Free hiPSCs
melanocytes that can be used for novel approaches for modeling human skin disease (e.g., melanoma and vitiligo) and to provide material for transplantation.

**CONFLICT OF INTEREST**
The authors state no conflict of interest.

**ACKNOWLEDGMENTS**

JCJ was supported by the California Institute for Regenerative Medicine (CIRM) Bridges to Stem Cell Research Internship Program (TB1-01177-1). Y-CW was supported by the Marie Mayer Foundation Postdoctoral Fellowship. JL and SEP were supported by grants from CIRM (RT1-01108, TR1-01250, and CL1-00362), NIH (R33MH087925), the Esther O’Keeffe Foundation, and the Millipore Foundation. MMP was supported by CIRM (RN2-00931) and NIH (1R01HD071100). YL was supported by CIRM (RT1-011071), the Memorial Hermann Foundation (Staman Ogilvie Fund), and Bentsen Stroke Center. LCL was supported by NIH/NICHHD Career Development Award (K12 HD001259). We thank Ms Nastaran Afari and Ms Kellen Na at University of California, San Diego, for their assistance in teratoma analysis.

Jennifer C. Jones1,2,3, Karen Sabatini1,2, Xiaoyan Liao1,2,4, Ha T. Tran1,2, Candace L. Lynch1,2, Robert E. Morey1,2, Victoria Glenn-Pratola1,2, Francesca S. Boscolo1,2, Qinghong Yang5, Mana M. Parast5, Ying Liu6,7, Suzanne E. Peterson1,2, Louise C. Laurent1,2,4, Jeannie F. Loring1,2 and Yu-Chieh Wang1,2

1Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California, USA; 2Center for Regenerative Medicine, The Scripps Research Institute, La Jolla, California, USA; 3California State University, Channel Islands, California, USA; 4Department of Reproductive Medicine, University of California, San Diego, San Diego, California, USA and 5Department of Pathology, University of California, San Diego, San Diego, California, USA; 6Current address: Department of Neurosurgery, University of Texas Health Science Center, Houston, Texas, USA

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

**REFERENCES**

Fang D, Leibehar K, Nguyen TK et al. (2006) Defining the conditions for the generation of melanocyte stem cells. Stem Cells 24:1668–77

Fistolar SK, Itin PH (2010) Disorders of pigmentation. J Dtsch Dermatol Ges 8:187–201

Fusaki N, Ban H, Nishiyama A et al. (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85:348–62

Lin JY, Fisher DE (2007) Melanocyte biology and skin pigmentation. Nature 445:843–50

Macarthur CC, Fontes A, Ravinder N et al. (2011) Generation of human-induced pluripotent stem cells by a nonintegrating RNA Sendai virus vector in feeder-free or xeno-free conditions. Stem Cells Int 2012:64612

Muller FL, Schuldt BM, Williams R et al. (2011) A bioinformatic assay for pluripotency in human cells. Nat Methods 8:315–7

Nissan X, Larribere L, Saidani M et al. (2011) Generation of human-induced pluripotent stem cells engraft into pluristratified epidermis. Proc Natl Acad Sci USA 108:14861–6

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

Pla P, Alberti C, Sokol’evaa O et al. (2005) Ednrb1 orients cell migration towards the dorsolateral neural crest pathway and promotes melanocyte differentiation. Pigment Cell Res 18: 181–7

Rees JL (2011) The genetics of human pigmentation disorders. J Invest Dermatol 131:E12–3

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

Thody AJ (1999) alpha-MSH and the regulation of melanocyte function. Ann NY Acad Sci 885:217–29

Wang YC, Nakagawa M, Garitaonandia I et al. (2011) Specific lectin biomarkers for isolation of human pluripotent stem cells identified through array-based glycomic analysis. Cell Res 21:1551–63

Yamane T, Hayashi S, Mizoguchi M et al. (1999) Derivation of melanocytes from embryonic stem cells in culture. Dev Dyn 216:450–8

Yang R, Jiang M, Kumar SM et al. (2011) Generation of melanocytes from induced pluripotent stem cells. J Invest Dermatol 131: 2458–66

---

**SUPPLEMENTARY MATERIAL**

Three-Dimensional Morphology of Touch Domes in Human Hairy Skin by Correlative Light and Scanning Electron Microscopy

Journal of Investigative Dermatology (2013) 133, 2108–2111; doi:10.1038/jid.2013.60; published online 18 April 2013

**TO THE EDITOR**

The presence of tactile structures in human hairy skin was first reported by Pinkus in 1902. He discovered distinct epidermal disc-like structures with nerves and “Tastzellen” (i.e., Merkel cells) at the base of the epidermis, and named these structures “Haarscheiben” because of their close association with hair follicles. These structures were revisited by later investigators (Kamide, 1955; Kawamura et al., 1964). Recent researchers have also reported the histology of the human “Haarscheiben” (Moll et al., 2005; Reinisch and Tschachler, 2005), whereas some other investigators showed that Merkel cells...