Biochemical and Biophysical Research Communications xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

In vitro and *in vivo* differentiation of induced pluripotent stem cells into male germ cells

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ARTICLE INFO

Article history:
Received 28 January 2013
Available online xxxx

Keywords:
Induced pluripotent stem cell (iPSC)
Germ stem cell (GSC)
Spermatogenesis
Niche
Ectopic reconstitution of seminiferous tubules

ABSTRACT

The introduction of induced pluripotent stem cell (iPSC) lines has been a breakthrough in the field of stem cell research. However, the extent of pluripotency among those cell lines tends to be variable due to their different epigenetic signatures. Mouse iPS cell line 4.1 has been established via retroviral transfer of human transcription factors Oct4, Sox2, Klf4, and c-Myc; the germline competence of this line has not been determined. In the present study, we induced the differentiation of miPS-4.1 cells into male germ cells, *in vivo* and *in vitro*. In the *in vitro* model, the behavior of miPS-4.1 cells was identical to that of differentiating mouse embryonic stem cells (ESCs). We obtained primordial germ cell-like cells (PGC-LC) that were positive for alkaline phosphatase (AP) activity. In continuous culture, these cells expressed pluripotent marker Oct4 and male germline markers C-kit and MVH. For our *in vivo* model, miPS-4.1 cells were co-transplanted with neonatal testicular cell suspension. We observed ectopically reconstituted seminiferous tubule structures, in which the miPS-4.1 cells were homing and developing. In conclusion, we successfully induced the differentiation of miPS-4.1 cells into male germ cells, albeit their epigenetic characteristics. Our study provides a system to examine the mechanisms of male germ cell development and might help to supply an effective treatment for male infertility in the future.

1. Introduction

Germ cell lineage transmits the genetic and epigenetic information across the generations, making possible the conservation of species. However, our knowledge of the germline specification is still limited; *in vitro* recreation of germ cell development has been one of the biggest challenges in modern biology. The *ex vivo* gametogenesis can be divided into two phases: the first is the differentiation from pluripotent cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), into the primordial germ cell-like cells (PGC-LCs); and the second, controlled initiation and completion of meiosis. These two phases are fundamentally independent, and different in males and females.

Much progress has been achieved in the derivation of male germ cells from ESCs. In mice, male gametes derived from these cells have been shown to produce viable offspring [1,2]. In humans, some successful methods for deriving male germ cells from ESCs have been also reported [3–5] although the ethical arguments have been inevitable. However, the induction of germ cell differentiation from iPSCs seems to be more difficult. In several studies, PGC-LCs have been generated from either mouse or human iPSCs [6–8]. Even post-meiotic male haploid cells have been obtained [6]; however, it remains to be shown whether these haploid cells are reproductively competent.

Unlike the studies of differentiation from ESCs, similar work based on iPSCs is likely to be affected by the original properties of iPS cell lines. The residual epigenetic memories carried by iPSCs might promote or impede the differentiating process. Mouse iPS cell lines miPSC-4.1 and miPSC-11.1 have been derived from the male mouse neural progenitor cells via induced reprogramming using retroviral transfer of human transcriptional factors Oct4, Sox2, Klf4, and c-Myc. However, miPSC-11.1 cells have been successfully induced to form contractile cardiomyocytes, while iPS cells from line 4.1 have failed to do so under the same conditions. On the other hand, miPSC- 4.1 cells differentiated into endoderm lineages more easily than miPSC-11.1 cells [9]. It has been proven that miPSC-11.1 cells can contribute to the germline in chimera mice, and some recent related studies have reported the differentiation of miPSC-11.1 cells into male germ cells [10,11]. By contrast, the germline competence of miPSC-4.1 cells has not been determined so far.

Abbreviations: iPSC, induced pluripotent stem cell; ESC, embryonic stem cell; EB, embryoid body; PGC, primordial germ cell; GSC, germ stem cell.

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0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.107

In the present study, we tried to induce miPSC-4.1 differentiation into male germ cells *in vitro* and *in vivo*. Using an *in vitro* protocol, we induced the iPSC-4.1cells into a PGC-like status. The resultant germ stem cell clones were characterized. At the same time, we developed a model of reconstituted seminiferous tubules, in which iPSC-4.1cells could properly differentiate into germ cells. In conclusion, we proved that the miPS-4.1 cell line has the male germline potential.

2. Materials and methods

2.1. Animals

Neonatal male ICR mice and adult male BALB/c-nu/nu mice were purchased from the Centre for Experimental Animals, Chinese Academy of Sciences, Shanghai, China. The animal procedures were conducted in accordance with the National Research Council Guide for Care and Use of Laboratory Animals [SYXK (Hu) 2008–0050)].

2.2. Mouse embryonic stem cell (mESC) and mouse induced pluripotent stem cell (miPSC) culture

The mESC-H1.2 (40, XY) and Tg-EGFP-miPSC-4.1 (40, XY) lines have been originally established by Dr. JIN's team (Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences/Shanghai Jiao Tong University). The mESC-H1.2 line has been generated from a male C57BL/6J mouse, while the miPSC-4.1line is derived from the neural progenitor cells of the EGFP-transgenic C57BL/6J male mouse. Cells were cultured as described previously [9].

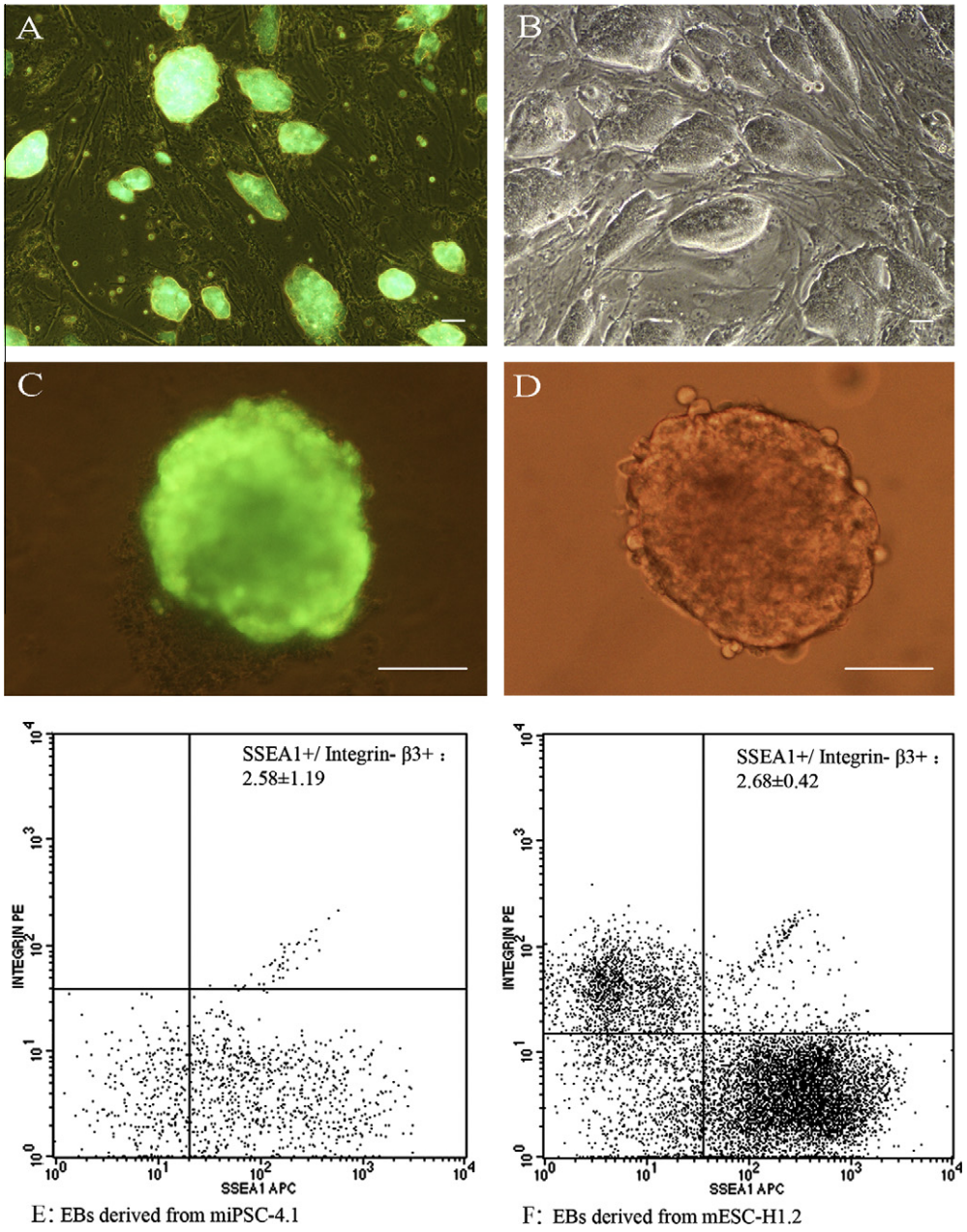

Fig. 1. Analyses of EBs derived from Tg-EGFP-miPSC-4.1 and mESC-H1.2 cell clones. (A–D) Morphology. (A) Tg-EGFP-miPSC-4.1 clones. (B) mESC-H1.2 clones. (C) 7-day EBs derived from miPSC-4.1. (D) 7-day EBs derived from mESC-H1.2. Green: (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) EGFP expression. Bars = 50 μm. (E–F) FCM analysis of derived EBs for SSEA-1 and integrin-β3 expression. (E) 7-day EBs derived from miPSC-4.1. (F) 7-day EBs derived from mESC-H1.2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: H. Cai et al., *In vitro* and *in vivo* differentiation of induced pluripotent stem cells into male germ cells, Biochem. Biophys. Res. Commun. (2013), http://dx.doi.org/10.1016/j.bbrc.2013.02.107