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Derivation of male germ cells from induced pluripotent stem cells by inducers: A review

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Abstract

Induced pluripotent stem cells (iPSCs) refer to stem cells that are artificially produced using a new technology known as cellular reprogramming, which can use gene transduction in somatic cells. There are numerous potential applications for iPSCs in the field of stem cell biology becauase they are able to give rise to several different cell features of lineages such as three-germ layers. Primordial germ cells, generated via *in vitro* differentiation of iPSCs, have been demonstrated to produce functional gametes. Therefore, in this review we discussed past and recent advances in the *in vitro* differentiation of germ cells using pluripotent stem cells with an emphasis on iPSCs. Although this domain of research is still in its infancy, exploring development mechanisms of germ cells is promising, especially in humans, to promote future reproductive and developmental engineering technologies. While few studies have evaluated the ability and efficiency of iPSCs to differentiate toward male germ cells *in vitro* by different inducers, the given effect was investigated in this review.

Key Words: *differentiation, germ cells, induced pluripotent stem cells, inducers*

Introduction

Currently, germ cell development *in vitro* is considered to be one of the biggest challenges in the domain of modern biology, and it is also known as one of the key goals in this respect [1]. There are two independent phases in *ex vivo* gametogenesis. The first phase consists of the differentiation of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), leading to primordial germ cell–like cells (PGCLCs). In the second phase, meiosis is initiated and completed [2].

The self-renewing and production capabilities of all cell types in a mammalian body are considered to be the main characteristics of pluripotent stem cells (PSCs) [3]. Moreover, at different stages of development, germ cells can give rise to PSCs. For example, spermatogonial stem cells (SSCs) and primordial germ cells (PGCs) are able to produce multipotent germline stem cells (mGSCs) and embryonic germ cells (EGCs), respectively [4,5].

Likewise, stem cells are a unique type of cells that are able to differentiate and self-renew; these include adult stem cells, iPSCs and ESCs. Recently, stem cells have been reported to have the ability to differentiate into germ cells provided that the appropriate conditions are met *in vitro*. New perspectives for regenerative and reproductive medicine also have been revealed by advances in this domain. Accordingly, in this article we reviewed the progress made in understanding how gametes are produced from stem cells [6].

Although successful methods have been reported on how ESCs can be differentiated into male germ cells in humans, there have been arguments about the ethics of doing so [7]. Several studies have similarly demonstrated that either human or mouse iPSCs are capable of producing postmeiotic male haploid cells

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and PGC-LCs [8–10]. As well, researchers have developed a new method to lead somatic origin substrates to become iPSCs [11].

Human-induced pluripotent stem (hiPS) cells obtained from the somatic cells of patients are expected to be a beneficial source for cell replacement therapy in comparison with other stem cells [12]. In the future, iPSCs will play an important role in mammalian development, diseases, pharmacological assessment and regenerative medicine. The ability of PSCs to differentiate into germ cells has been also examined in several species including mice, chickens and several primates such as cynomolgus monkeys and humans [3]. Moreover, human embryonic stem cells (hESCs) have been demonstrated to differentiate into male and female cells, but more studies must be conducted to show the progression ability of iPSCs through meiosis [10]. It is probably more difficult to induce germ cell differentiation from iPSCs, therefore, the capacity of hiPS cells to generate human germ cells *in vitro* has not yet been evaluated [8]. With particular attention given to recent studies, it is important to estimate the quality of each undifferentiated iPSC clone rigorously on the basis of both its characteristics and its differentiation power [13,14].

Furthermore, important platforms might be provided through new advances in male germ cell differentiation *in vitro* by different inducers of human disease, drug testing, and novel reproductive technologies (Table I). In this review, the derivation of iPSCs into male germ cells by different inducers was compared.

iPSCs

The generation of iPSCs from somatic cells is considered to be a very recent development; however, it plays a profound role in clinical therapy [23,24]. When a specific group of reprogramming factors is introduced into somatic cells, iPSCs are produced and they are believed to be an efficient cell source in the domain of cell therapy [25].

In 2007, Takahashi *et al.* demonstrated that reprogramming human somatic cells to an embryonic stem cell–like pluripotent state was accomplished through key transcription factors that were forced to express [26]. It was immediately shown that such cells can be produced from patient-specific cells for several different states of a disease [27] and from various types of somatic cells (Figure 1) [28,29].

One of the benefits of generating and using iPSCs, especially for autologous stem cell therapy, is that doing so poses fewer ethical problems than the derivation and use of ESCs. There are also small differences in the gene expression patterns between these cells and embryonic stem cell–like cells [30]. The methods used to generate these cells for clinical applications are very controversial; the growth and developmental characteristics of these cells as well as their clinical utility may be jeopardized by the use of lentiviral and retroviral vectors and the techniques used for reprogramming diseased cells and oncogenes such as c-Myc and KLF4 with low efficiency [31]. The differentiation ability of iPSCs and hESCs is not the same, and, the teratoma-formation properties of iPSCs derived

Table I. Overview of *in vitro* germ cell differentiation studies in humans and mice.

References	Source cells (gender)	Culture methods	*In vitro*–derived cells
Imamura *et al.* (2010) [14]	Mouse iPSCs (XY)	EBs (BMP4, EGF, GDNF, SCF-producing cells)	PGCs, oocytes
Hayashi *et al.* (2012) [15]	Mouse ESCs (XX), Mouse iPSCs (XX)	EpiLCs and PGCLCs induction	PGCs
Yang *et al.* (2012) [16]	Mouse iPSCs (XY)	EBs (RA)	Male germ cells
Zhu *et al.* (2012) [17]	Mouse iPSCs (XY)	EBs (RA)	Male germ cells
Eguizabal *et al.* (2011) [9]	Human ESCs (XY, XX), Human iPSCs (XY, XX)	Adherent culture (RA, etc.)	PGCs, spermatids
Easley *et al.* (2012) [18]	Human ESCs (XY), Human iPSCs (XY)	Adherent culture (GSC condition)	SSCs, spermatocytes, spermatids
Medrano et al. (2012) [19]	Human ESCs (XY, XX), Human iPSCs (XY, XX)	Adherent culture (VASA/DAZL over expression)	Spermatids
Park *et al.* (2009) [8]	Human ESCs (XY) Human iPSCs (XY)	Co-culture with human fetal gonadal cells	PGCs
Panula *et al.* (2011) [10]	Human iPSCs	BMPs	PGCs
Li *et al.* (2013) [20]	Mouse iPSCs (XY)	EB formation and RA/testosterone induction	Male germ cells
Yang *et al.* (2017) [21]	Mouse iPS cells	BMP-4	Male germ cells
Wang *et al.* (2016) [22]	Porcine iPS cells	EB formation and RA, GDNF, and testosterone	Male germ cells

EBs, embryoid bodies; BMP4, bone morphogenetic protein 4; EGF, epidermal growth factor; GDNF, glial cell-derived neurotrophic factor; SCF, stem cell factor; EpiLCs, epiblast-like cells; RA, retinoic acid.