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Review

Derivation of porcine pluripotent stem cells for biomedical research

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A B S T R A C T

Pluripotent stem cells including embryonic stem cells (ESCs), embryonic germ cells (EGCs), and induced pluripotent stem cells (iPSCs) are capable of self-renew and limitlessly proliferating *in vitro* with undifferentiated characteristics. They are able to differentiate *in vitro*, spontaneously or responding to suitable signals, into cells of all three primary germ layers. Consequently, these pluripotent stem cells will be valuable sources for cell replacement therapy in numerous disorders. However, the promise of human ESCs and EGCs is cramped by the ethical argument about destroying embryos and fetuses for cell line creation. Moreover, there are still carcinogenic risks existing toward the goal of clinical application for human ESCs, EGCs, and iPSCs. Therefore, a suitable animal model for stem cell research will benefit the further development of human stem cell technology. The pigs, on the basis of their similarity in anatomy, immunology, physiology, and biochemical properties, have been wide used as model animals in the study of various human diseases. The development of porcine pluripotent stem cell lines will hold the opportunity to provide an excellent material for human counterpart to the transplantation in biomedical research and further development of cell-based therapeutic strategy.

1. Introduction

Pluripotent stem cells are cell lines established from embryos including embryonic stem cells (ESCs) and epiblast stem cells (EpiSCs) or from the primordial germ cells (PGCs) in the genital ridge of fetus as embryonic germ cells (EGCs) [1]. Also, from somatic cells reprogrammed by ectopic coexpression of various defined transcription factors [2] are approached to generate pluripotent cells, which possessed comparable characteristics of ESCs and nominated as induced pluripotent stem cells (iPSCs; Fig. 1).

Embryonic stem cells are firstly isolated from preimplantation mouse embryos [3,4]. Thereafter, isolation of putative pluripotent ESC lines other than murine ES cells (mESCs) has been attempted in various mammalian species (refers to review in [5]), including human [6].

Defined ESCs are able to self-renew and proliferate continuously *in vitro* with the undifferentiated characteristics. In responding to suitable conditions, they can be induced to differentiate into cells of all three primary germ layers. There were many remarkable results in directing the human ES cells (hESCs) differentiation into neuronal cells

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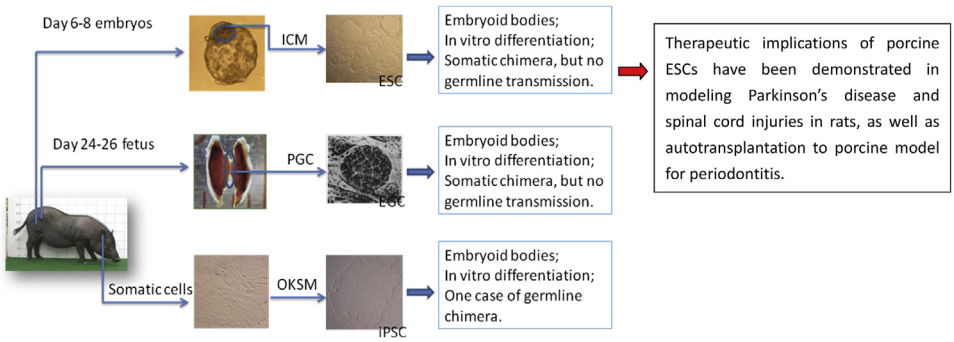

Fig. 1. Generation of porcine pluripotent cell lines. Porcine ESC and EGC lines can be derived from embryos and genital ridges of fetuses at Days 6 to 8 and 24 to 26 of fertilization, respectively, of gestation. These cell lines can form embryoid bodies and differentiate into cell lineages representative to three prime germ layers. Offspring of somatic but not germline chimera has been obtained by blastocyst injection of porcine ESCs and EGCs, respectively. Porcine iPSCs can be generated from somatic cells by reprogramming with transcription factors of OKSM or Yamanaka factors. These cells are proven to be pluripotent because they have resulted in chimeric offspring with germline transmission. EGC, embryonic germ cell; ESC, embryonic stem cell; ICM, inner cell mass; iPSCs, induced pluripotent stem cells; PGC, primordial germ cell.

[7], pancreatic β cells [8], and cardiomyocytes [9]. These results reveal a potential for clinical application of hESCs in the treatment of diseases such as Parkinson's disease, spinal cord injure, diabetes, and heart diseases.

Domestic swine are demonstrated to be very similar to the human in anatomic, immunologic, and physiological characteristics, and the sizes of their organs are fairly comparable to those of human. Moreover, swine, especially the miniature pigs, has been demonstrated as excellent animal model in therapeutics development for various human diseases, including congenital heart disease, hypertension, organ transplantation, pharmacology, and toxicology [10,11]. Therefore, the study of porcine pluripotent stem cells might serve as an excellent model in development of biomedical and regeneration medicine in humans [12].

2. Derivation of porcine ESC (pESC) lines from embryos of different origins

Although the establishment of ESC lines from domestic species is much more difficult than that in murine species [5,13,14], putative pESCs have been derived from inner cell mass (ICM) of the *ex vivo* blastocysts, *in vitro*-produced blastocysts, parthenogenetic blastocysts, and the blastocysts derived from somatic cell nuclear transplantation with diverse derivation efficiency [5,14,15]. In general, almost all the blastocyst-derived putative pESC lines possessed an epithelial-like morphology, were feeder-dependent, and expressed alkaline phosphatase (AP). However, the capability of these blastocyst-derived cells in self-renew, proliferation, maintenance of undifferentiated status *in vitro*, and pluripotency is varied among the cell lines reported, and most of them lose their pluripotency subsequently [5,12,15]; if any which retain the capacity of repeated proliferation and pluripotency, they possess very limited capacity in chimera generation [16,17], and so far, no germline transmissions are obtained when injected into a blastocyst (Table 1).

The pESCs were very similar to hESCs in many characteristics, including colony morphology, feeder-dependent, and refractory to leukemia inhibitory factor (LIF) in culture, and expression of stem cell markers [13,15,16,22]. To our knowledge, isolation and establishment of persistent pESC lines were firstly accomplished and reported by our laboratory in 1999 by adapting the ESC culture system for murine species, with minimum modification [16]. Briefly,

Table 1
Summary of the main results describing ESCs derivation from pig embryos for more than 20 passages.[a]

Embryo origin	d.p.i.	Maximum passage number	Method of undifferentiation evaluation	Method of evaluation and type of differentiation	Reference
Ex vivo	7 or 9	>1 y; >50 passages	Morphology, vimentin	Morphology, EB, muscle, nerve, and endoderm	[13]
Ex vivo	7–8	>80 passages, two male lines	Morphology	Morphology, neuron, muscle, epithelium, adipocytes, melanocytes, and glandular epithelium	[18]
Ex vivo	5.5–7.5	44 passages	Morphology	Morphology: EB, adipocytes, epithelium, neurons, muscles cells, EB, and chimera	[19]
Ex vivo	6–8	>35 passages, one male, three female lines	Morphology, AP	Morphology: neuron, smooth muscle, epithelium, EB, and somatic chimera	[16]
Ex vivo	6–8	One male line	Oct-4, AP, SSEA-3/4, TRA 1–60, and TRA 1–81	EB, neuron, and used for cell transplantation	[20]
In vitro	7–8	One line, 30 passages	Morphology, epithelial-like	Used for nuclear transfer with embryo development to blastocyst stage	[21]

Abbreviations: AP, alkaline phosphatase; d.p.i., day post insemination; EB, embryoid body; ESCs, embryonic stem cells.
[a] Modified from Brevini et al., Theriogenology 2010; 74: 544–550.