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Extended Abstract

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Induced pluripotent stem cells derived from porcine Sertoli cells

P Setthawong

Chulalongkorn University, Thailand

E-mail: piyathip.ps@gmail.com

Induced pluripotent stem cells (iPSCs) are generated by reprogramming of fully differentiated adult cells using four transcription factors, including OCT4, SOX2, KLF-4 and c-MYC (OSKM). Porcine is a meaningful model for regenerative medicine because its anatomy and physiology are similar to human. However, reprogramming efficiency of porcine fibroblasts into iPSCs is currently poor. This study used Sertoli cells as a novel cell origin for somatic cells reprogramming. Neonatal testes were collected from 1-week old piglets. The testes were digested by two-step enzymatic method in order to isolate the Sertoli cells. The Sertoli cells were transfected with retroviral vectors expressing OSKM. We observed the primary colonies and counted on day 7 after transfection. The characteristics of Sertoli iPSCs-like colonies were analyzed by morphology, alkaline phosphatase staining, RTPCR, G-banding, in vitro and in vivo differentiation. Sertoli cells obtained from neonatal porcine showed typically polygonal shaped. A total of 240 colonies (0.33%) originated from seeding 72,500 cells were observed on day 7. The Sertoli iPSCs-like colonies exhibited a high nuclear per cytoplasm ratio with prominent nucleoli. We picked up 30 Sertoli iPSCs-like colonies and 8 cell lines (26.6%) demonstrated undifferentiated stage of iPSCs. The Sertoli iPSCs like colonies were positive to alkaline phosphatase staining and expressed endogenous pluripotent. G-banding analysis demonstrated normal karyotype. Under differentiation conditions, iPS-like cell lines could form three-dimension aggregated masses, which represented three germ layers of embryonic cells. For in vivo differentiation, tumour mass were collected and presented all of ectoderm, mesoderm, and endoderm. In conclusion, the Sertoli cells can be used as a novel somatic cell origin for iPSCs reprogramming. Induced pluripotent stem cells (iPSCs) are laboratory-developed pluripotent stem cells generated by the reprogramming of differentiated cells. Takahashi and Yamanaka first discovered somatic cells' capacity for reprogramming in 2006 after forcing differentiated fibroblast cells to ectopically express four transcription factors associated with pluripotency: Oct4, Sox2, Klf4, and c-Myc, collectively referred to as OSKM. iPSCs have since been of interest to researchers in the fields of toxicology, pathology, virology, developmental anatomy and physiology, amongst others. iPSCs possess several benefits over other stem cell types such as mesenchymal stromal cells (MSCs) and embryonic stem cells (ESCs). In the context of this review, the term mesenchymal *stromal* cells has been adopted over mesenchymal *stem* cells due to the finite self-renewing property of MSCs that does not support the traditionally recognized self-renewing characteristic of stem cells. The versatility of iPSCs may make them preferential over MSCs that are limited in their differentiation potential due to their multipotent nature. ESCs offer a similar versatility to iPSCs as they are both pluripotent, but not without limitations. ESCs can be obtained from in vivo and in vitro produced embryos at the blastocyst stage. However, technical difficulties have interfered with the isolation and use of ESCs, namely in ungulate species and canines. Oocyte collection for in vitro embryo production is an invasive procedure that has prompted ethical considerations. Disposed reproductive material has been the primary source of oocytes in domestic species obtained from meat processing in livestock or ovariohysterectomies in companion animals. In vivo protocols may include minimally invasive uterine flushing, often seen in mares. iPSCs provide a more practical alternative to creating ESC-like cells in species where recovery of embryos or in vitro fertilization is difficult or not possible. Unlike ESC lines, autologous iPSC lines can also be produced. This is ideal for transplantation of stem cells and their derivatives as it avoids the immunological complications associated with allogeneic iPSCs. Consequently, iPSCs can be used as an alternative to MSCs and ESCs with the potential for greater research and clinical applicability in domestic species. While research has focused primarily on human and mice iPSCs, there has been a slow accumulation of iPSC research in domestic animals in the last decade. iPSC derivation protocols have been developed in species including porcine, equine, canine, bovine, galline, caprine, ovine, and feline. Aside from their importance in treating veterinary pathologies, porcine, canine, and equine models have been shown to be valuable for the study and treatment of human disease. The purpose of this review is to provide an overview of the literature pertaining to current protocols and applications of iPSCs derived from domestic species. This review will address the topics of the development and use of iPSCs for tissue and disease research, their treatment in domestic animals and the barriers to their production and applications. iPSCs have been produced from various donor tissue types, transduction systems, and reprogramming factor combinations. In domestic species, iPSCs have been derived from fibroblasts, MSCs and other somatic cell types including epithelial and testicular cells. Tissue sources have been obtained from various developmental stages, namely fetal, neonatal, juvenile, and adult. For simplicity, this review has identified any tissue sources obtained from an animal in utero as fetal and those obtained after birth as adult. Deriving iPSCs from adult somatic cells is generally preferable to embryonic derivation due to a higher abundance of cells, easier collection of cells, and the ability to produce autologous iPSC populations for disease treatment. Donor tissue is then cultured and reprogrammed using viral or non-viral vectors containing the designated reprogramming factors. Viral vectors include lentiviruses, oncoviruses, and Sendai viruses, while non-viral vectors include cDNA vectors, minicircles and transposons. The selected reprogramming factors typically include OSKM, but other variations have also been explored. Nanog and Lin28 are commonly used in the literature in addition to OSKM, and a small number of papers report the use of other additional transcription factors, such as TERT, and Tet1. More recently, work has been carried out using microRNAs in combination with other factors to achieve pluripotency induction. MicroRNAs alone have only shown partial reprogramming abilities in domestic animals.

Following presumed iPSC production, colonies can be analyzed via morphological assessment to select for colonies with the most potential in reprogramming cells to an undifferentiated state. Non-invasive morphological assessment also provides insight into the developmental competence and homogeneity of iPSC colonies. Traditionally, iPSC colonies resemble ESC colonies with well-defined borders and tightly packed cells. More specifically, dome-shaped and flattened colonies are indicators of naïve and primed pluripotency, respectively. Cells in these colonies are expected to have a large nucleus and little cytoplasm. Naïve pluripotency is recognized by characteristic molecular features of the pre-implantation mouse embryonic stem cell, whereas primed pluripotency resembles stem cells of the post-implantation mouse epiblast. Naïve pluripotent stem cells are identified by X chromosome reactivation in females, dependency on leukemia inhibitory factor (LIF) and receptivity to BMP4 to maintain pluripotency, and the transition to a more differentiated state in response to FGF2 and ACTIN/TGFB signalling. Putative iPSCs must then undergo a series of tests to confirm pluripotency. In domestic species, pluripotency is often confirmed by the endogenous expression of pluripotency markers, and the formation of in vitro embryoid bodies and in vivo teratomas containing cell types derived from all three germ layers. Chimera formation with germ-line transmission is a less commonly used method in domestic species, but is deemed the gold standard for validating stem cell pluripotency.

Bottom Note: *This work is partly presented at* EuroSciCon Joint Event on Biotechnology, Stem Cell and Molecular Diagnostics April 16-17, 2018 Amsterdam, Netherlands.