Animal Reproduction Science 147 (2014) 39–46

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Passage number of porcine embryonic germ cells affects epigenetic status and blastocyst rate following somatic cell nuclear transfer

Juan Li[a,*], Yu Gao[b,1,2], Stoyan Petkov[c], Stig Purup[d], Poul Hyttel[b], Henrik Callesen[d]

[a] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing Weigang No. 1, Jiangsu Province, China
[b] Department of Basic Animal and Veterinary Sciences, University of Copenhagen, Denmark
[c] Institute for Farm Animal Genetics, Neustadt, Germany
[d] Department of Animal Science, Aarhus University, Tjele, Denmark

ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form 18 February 2014
Accepted 16 March 2014
Available online 1 April 2014

Keywords:
Nuclear transfer
Methylation
Acetylation
In vitro

ABSTRACT

Epigenetic instability of donor cells due to long-term *in vitro* culture may influence the success rate of subsequent somatic cell nuclear transfer (SCNT). Therefore, the present study was designed (1) to investigate the epigenetic changes after prolonged culture *in vitro* of porcine embryonic germ (EG) cells, including differences in expression levels of both DNA methylation and demethylation-related genes and catalyses of histone modifications, and (2) to assess the efficiency of SCNT using EG cells from different passages. Results showed that genes either associated with DNA demethylation including DNMTs and TET1 or genes related to histone acetylation including HDACs were highly expressed in EG cells at higher passages when compared to EG cells at lower passages. In addition, the expression level of H3K27me3 functional methylase EZH2 increased while no changes were observed on H3K27me3 demethylase JMJD3 in relation to passage number. Moreover, the expression levels of both the H3K4me3 methylase MLL1 and the H3K4me3 demethylase RBP2 were increased at high passages. By using lower passage (numbers 3–5) EG cells as donor cells, the SCNT efficiency was significantly lower compared with use of fetal fibroblast donor cells. However, similar blastocyst rates were achieved when using higher passage (numbers 9–12) EG cells as donor cells. In conclusion, the present study suggests that the epigenetic status of EG cells change with increasing passage numbers, and that higher passage number EG cells are better primed for SCNT.

1. Introduction

Somatic cell nuclear transfer (SCNT, "cloning") has wide applications in high-quality domestic animal reproduction, basic biology as well as stem cell and human disease studies. The cloning inefficiencies may be attributed to many factors that are not fully understood, but the state of DNA in the donor cells (e.g. inappropriate nuclear status, incomplete reprogramming, chromosomal abnormalities, damaged DNA) has been suggested as one factor (Ahn et al.,

* Corresponding author at: Nanjing Weigang No. 1, Jiangsu Province 210095, China. Tel.: +86 84395278; fax: +86 025 84395314.
E-mail address: juanli@njau.edu.cn (J. Li).
[1] This author contributed the same as the first author.
[2] Current address: Waisman Center, University of Wisconsin-Madison, WI, USA.

http://dx.doi.org/10.1016/j.anireprosci.2014.03.012
0378-4320/

2007; Yang et al., 2012; Tian et al., 2012). Evidence has indicated that failure of nuclear transfer embryos to develop normally can be attributed, at least partially, to the use of a differentiated cell nucleus as the donor karyoplast, and embryonic stem or germ cells have therefore been investigated as possible useful alternatives to somatic cells. In addition, cell passage affected the levels of H3K9ac in bovine fibroblasts, and further reduced cleavage and blastocyst rates (Chacóon et al., 2011). Embryonic germ (EG) cell derivation has been reported in pig, and such EG cells are multipotent rather than are pluripotent in the pig and should, hence, be referred to as EG-like cells (Tsung et al., 2003; Petkov et al., 2009, 2011).

In vitro conditions may provoke changes of the epigenetic modifications that regulate chromatin compaction and gene expression in cultured cells (Enright et al., 2003). Such changes will affect the ability of the cells to undergo reprogramming during SCNT. Shortly before and after the formation of the cloned embryos, the nucleus responds to the molecular factors in the cytoplast by the epigenetic reprogramming of genomic DNA modifications (Condic, 2008; Deshmukh et al., 2011). The epigenetic status of the donor cells might be valuable in assessing the potential success of subsequent SCNT (McLean et al., 2010). Indeed, DNA methylation critically depends on the activity of a class of enzymes, the DNA methyltransferases (DNMTs). Based on the state of the substrate DNA double strands, DNMTs have been categorized into two main classes: (1) Those in charge of the establishment of *de novo* DNA methylation (e.g. DNMT3A or DNMT3B) and (2) DNMT1 and its isoforms, which can only methylate hemi-methylated DNA and is responsible for DNA methylation maintenance (Bestor, 2000; Li, 2002). In addition to DNA methylation, chromatin compaction is regulated by histone acetylation, while histone deacetylases (HDACs) are responsible for removal of acetyl groups from histone tails, which leads to gene repression (Grunstein, 1997; Imhof et al., 1997). Therefore, HDACs are considered as the transcriptional co-repressors. Actually, DNMTs and HDACs (HDAC1 and HDAC2) have been well studied in mammals (Montgomery et al., 2007; Zimmermann et al., 2007; Gao et al., 2011) for their modulation of the chromatin structure. Evidence indicates that DNMTs and HDACs play essential roles on either DNA methylation or histone acetylation during embryogenesis (Enright et al., 2003; Dovey et al., 2010; Haberland et al., 2009). In addition, recent studies have shown that ten-eleven translocation (TET) proteins including TET1 can mediate DNA demethylation by catalyzing 5mC oxidation and generating 5mC derivations (Tahiliani et al., 2009; Ito et al., 2011; He et al., 2011). Hence, in the present study, we investigated the expression of DNMT1, DNMT3A, DNMT3B, HDAC1, HDAC2 and TET1 in different passage populations of EG cells in pig and related this to the efficiency of producing cloned embryos after use of EG cells as donor cells.

Actually, histone acetylation and DNA methylation are dynamic processes that establish a combinatory histone code which governs the chromatin configuration (Rice and Allis, 2001), that have an essential role in both transcriptional repression and activation during embryonic development (Surani et al., 2007). It has been reported that trimethylation of H3 at lysine 4 (H3K4) and lysine 27 (H3K27) are crucial factors in response to environment cues (van Dijk et al., 2010; Gao et al., 2011; Du et al., 2013). However, as the functional methylase (Cao and Zhang, 2004; Pasini et al., 2004; Montgomery et al., 2005), enhancer of zeste homolog 2 (EZH2) and mixed lineage leukemia 1 (MLL1) can catalyze the trimethylation of H3K27 and H3K4, respectively. Conversely methyl residues are not only deposited at specific lysines by methylases, but are also removed by specific demethylases. Jumonji domain containing 3 (JMJD3) can be responsible for the demethylation of H3K27me3 (Agger et al., 2007; Hong et al., 2007), and retinol binding protein 2 (RBP2) has recently been identified as a demethylase of H3K4me3 (Christensen et al., 2007).

Therefore, the expression levels of genes orchestrating epigenetic regulations were examined in porcine EG cells cultured without feeder layers at different passage numbers, and, subsequently, the developmental competence was tested of cloned embryos produced from EG donor cells from either low or high passage numbers.

2. Materials and methods

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise stated.

2.1. Cell preparation and in vitro culture

We have previously reported the establishment of EG cell lines and the analyses of their characteristics (Petkov et al., 2011). Briefly, hindguts from Yucatan Day 17 to 18 embryos I only know it was from Day 17 to 18 embryos, but not know the number of embryos being used) were isolated, while the medial parts of the mesonephros including the areas of genital ridge formation were dissected. The tissues were disaggregated by trypsinization, and the cell suspensions were cultured on mouse STO feeder layers with EG cell culture medium (AQ Media supplemented with 17% knockout serum replacement, nonessential amino acids, penicillin-streptomycin, 10 ng/ml human recombinant LIF (Millipore, Billerica, USA), 3 ng/ml human recombinant bFGF (Invitrogen, Carlsbad, USA), 10 ng/ml human recombinant SCF (Prospec, Rehovot, Israel)). To purify these primary cells, the cultured colonies were disaggregated by culturing with 1 mg/ml Collagenase IV for 10 min at 37 °C before being filtered three times through a 23 μm polyester mesh (Spectrum Labs, Los Angeles, USA). The putative EG cell colonies (which were very tightly packed and could not be disaggregated by the collagenase treatment) were recovered by washing the mesh with PBS, before they were disaggregated by trypsin and then split onto fresh feeders. After six passages, EG cell colonies were frozen and kept in liquid nitrogen until further use. Two EG cell lines were established separately in the same way which were defined as I and II.

After thawing the EG cells (passage designated as P1) were cultured *in vitro* without feeder cells for 4–6 days to 90–100% confluence in wells of 4-well dishes (NUNC A/S, Roskilde, Denmark) in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Life Technologies, New York, USA) supplemented with 1% non-essential amino acids, 2 mM