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Association between mitochondrial DNA haplotype compatibility and increased efficiency of bovine intersubspecies cloning

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Abstract

Reconstructed embryos derived from intersubspecies somatic cell nuclear transfer (SCNT) have poorer developmental potential than those from intrasubspecies SCNT. Based on our previous study that Holstein dairy bovine (HD) mitochondrial DNA (mtDNA) haplotype compatibility between donor karyoplast and recipient cytoplast is crucial for SCNT embryo development, we performed intersubspecies SCNT using HD as donor karyoplast and Luxi yellow heifer (LY) as recipient cytoplast according to mtDNA haplotypes determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The results demonstrated that intersubspecies mtDNA homotype SCNT embryos had higher pre- and post-implantation developmental competence than intrasubspecies mtDNA heterotype embryos as well as improved blastocyst reprogramming status, including normal H3K9 dimethylation pattern and promoter hypomethylation of pluripotent genes such as *Oct4* and *Sox2*, suggesting that intersubspecies SCNT using LY oocytes maintains HD cloning efficiency and may reprogram HD nuclei to develop into a normal cloned animal ultimately. Our results indicated that karyoplast−cytoplast interactions and mtDNA haplotype compatibility may affect bovine intersubspecies SCNT efficiency. This study on bovine intersubspecies SCNT is valuable for understanding the mechanisms of mtDNA haplotype compatibility between karyoplast and cytoplast impacting the bovine SCNT efficiency, and provides an alternative and economic resource for HD cloning.

Keywords: Somatic cell nuclear transfer (SCNT); Mitochondria; mtDNA haplotype; Epigenetic modification; Bovine; Intersubspecis; Intrasubspecis; Developmental competence

1. Introduction

Somatic cell nuclear transfer (SCNT) is a powerful tool for cloned animal production and developmental biology research. Reprogramming of the donor nucleus by the recipient oocyte is crucial during early development of the reconstructed embryo (Hiendleder et al., 2004), but the mechanism remains unclear. Generally, the efficiency of SCNT is low (Wilmut et al., 2002) and a number of studies have focused on improving the efficiency of bovine cloning (Betthauser et al., 2006; Fujii et al., 2010; Monteiro et al., 2010). Our previous study showed that usage of donor cells and enucleated oocytes from an individual female bovine, termed "autologous SCNT", improved the cloning efficiency and resulted in a decrease in reprogramming deficiencies compared with the "allogeneic SCNT" procedure, which utilizes donor cells and enucleated oocytes from different females (Yang et al., 2006). Because mitochondria are the most abundant organelle in the oocyte, they are considered important for the karyoplast−cytoplast interaction during reprogramming of the cloned embryo (Smith et al., 2004). Using a polymerase chain reaction-restriction fragment length polymorphism

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(PCR-RFLP) procedure, bovine mitochondrial DNA was identified and classified into two different major haplotypes, and homotype SCNT and heterotype SCNT (karyoplast donor and oocyte recipient have the same or different mitochondrial DNA haplotypes, respectively) were performed, resulting in an efficient method for bovine cloning (Yan et al., 2010). Based on the observation that nucleotide variations were lower among bovines of the same haplotype, regardless of breed (Jiao et al., 2007), we assumed that intersubspecies cloning using the homotype SCNT procedure might be more efficient than intrasubspecies heterotype SCNT. Aiming at exploring mtDNA haplotype compatibility between karyoplast and cytoplast in bovine intersubspecies cloning, we performed the cloning experiment with Holstein dairy bovine (HD) donor cells paired with oocytes derived from Luxi Yellow (LY) heifers (a subspecies of bovine) with specific mtDNA haplotypes. The results indicated that the homotype SCNT groups had higher pre- and post-implantation developmental potential as well as less epigenetic defect, no matter with the intersubspecies/intrasubspecies SCNT experiments. Our study is of great value for understanding the mechanisms of mtDNA haplotype compatibility between karyoplast and cytoplast impacting the bovine intersubspecies SCNT efficiency.

2. Materials and methods

All chemicals and media were purchased from Sigma−Aldrich (Sigma−Aldrich, USA) unless otherwise indicated.

2.1. Animals

In total, 2104 healthy heifers (1635 Holstein dairy heifers and 469 Luxi yellow heifers, 12−17 months old) with normal weight were used for ovum pick up (OPU). All animals were provided by the Songjiang Experimental Animal Facility, which is affiliated with the Shanghai Institute of Medical Genetics, P. R. China, and were housed in barns and fed a mixed ration consisting of hay and a commercial concentrate.

2.2. mtDNA haplotypes

DNA extraction from white blood cells of 2104 bovines was performed as described (Brown et al., 1989). Four pairs of primers (Table 1) were designed and utilized for PCR amplification of bovine mtDNA fragments (defined as H1, H2, H3 and H4) as previously described (Jiao et al., 2007) with slight modifications. PCR amplification was followed by restriction fragment length polymorphism (RFLP) analysis. Six enzymes (*Nla* III for H1, *Hpa* II for H1, *Hpa* II for H2, *Pst* I for H2, *Ava* II for H3, *Bam*H I for H3, and *Bgl* II for H4) were selected for haplotyping mtDNA. Restriction digestions were performed according to the manufacturer's recommendations (New England Biolabs, USA). Restriction fragments of each PCR product were analyzed by agarose gel electrophoresis.

2.3. Ovary recovery and in vitro maturation (IVM)

Heifers of two subspecies identified by mtDNA haplotyping to have one of two major haplotypes (A-haplotype and B-haplotype) were subjected to OPU as described previously (Yang et al., 2005). Collected cumulus−oocyte complexes (COCs) were categorized into three groups (grade A, B and C) as previously described (Yang et al., 2005). All COCs were matured for 19−20 h at 38.5 °C in IVMD (Fujihira, Japan) consisting of 10% FBS (fetal bovine serum, HyClone, USA) and 1% PS (Penicillin−Streptomycin, Invitrogen, USA) under 5% CO_2 and a humidified atmosphere.

2.4. Preparation of bovine fibroblasts as nuclear donors

Fibroblast cells were isolated from the superficial layers of the ear of Holstein dairy heifers with mitochondria DNA haplotype A and washed three times with PBS containing 5% antibiotic−antimycotic (Invitrogen), followed by a wash with PBS solution. The prepared fibroblast cells were cultured in 5 mL DMEM/F12 (Invitrogen) supplemented with 10% FBS at 38.5 °C under 5% CO_2 in a humidified atmosphere. The cells

Table 1
PCR primers for PCR-RFLP analysis of mtDNA haplotype and bisulfite conversion sequencing analysis.

Primers	Orientation	Primer sequence (5′→3′)	PCR product size (bp)
H1	Upstream	CTGCAGTCTCACCATCAACC	1094
L1	Downstream	GTGTAGATGCTTGCATGTGTAAGT	
H2	Upstream	TTATCCGTTGGTCTTAGGAA	4119
L2	Downstream	GCGGCATGGTAATTAAGCTC	
H3	Upstream	TTATCACAATCCAGAACTGAC	3910
L3	Downstream	CTAGTGAGAGTGAGGAGAATATG	
H4	Upstream	TGTGCATGTGACACGTATCC	2329
L4	Downstream	TTCCGGTCTGTTAATAGCATTG	
Oct4	Upstream	ATTTGGATGAGTTTTTAAGGGTTTT	292
	Downstream	ACTCCAACTTCTCCTTATCCAACTT	
Sox2	Upstream	TTTTTTAATTATAATTTGATGGGGT	288
	Downstream	CTAACACACCTTAAATAAACAAACC	
Nanog	Upstream	GGGATATGATTAGTATGTATTTTTT	230
	Downstream	TTCTCCATACTATTTCTTACTATCCTCC	

'H' and 'L' indicate the heavy strand and light strand of mtDNA, respectively.