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Effect of donor cell type on developmental competence, quality, gene expression, and epigenetic status of interspecies cloned embryos produced using cells from wild buffalo and oocytes from domestic buffalo

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ABSTRACT

This study compared the cloning efficiency of donor cells of fibroblast and epithelial origin isolated from ear skin of a wild buffalo (*Bubalus arnee*) and used with cytoplasts from domestic buffalo (*Bubalus bubalis*) in interspecies SCNT by hand-made cloning. The cleavage (93.0 ± 2.8% vs. 85.6 ± 2.4%) and blastocyst rates (50.6 ± 4.0% vs. 20.5 ± 2.6%) were higher ($P < 0.05$) for fibroblasts than those for epithelial cells, whereas the total cell number (490 ± 42 and 492 ± 95, respectively) and apoptotic index (2.3 ± 0.3 and 2.5 ± 0.6, respectively) of blastocysts were similar. The global level of H3K18ac and H3K27me3 was lower ($P < 0.05$) in fibroblasts than that in epithelial cells. The global level of H3K18ac was higher ($P < 0.05$) in fibroblast than that in epithelial cell–derived blastocysts, whereas that of H3K27me3 was similar between the two groups. The expression level of *HDAC1*, *DNMT1*, *DNMT3a*, and *P53* was higher ($P < 0.05$) in fibroblasts than that in epithelial cells; that of *CASPASE3* showed an opposite pattern ($P < 0.001$), whereas *CASPASE7* expression level was similar in the two groups. In the embryos, the expression level of *HDAC1*, *DNMT3a*, and *CDX2* was lower ($P < 0.05$) in fibroblast than that in epithelial cell–derived blastocysts; that of *NANOG* showed an opposite pattern ($P < 0.05$), whereas that of *OCT4* was similar between the two groups. In conclusion, donor cells of fibroblast origin are easier to reprogram than those of epithelial origin in interspecies SCNT, and cloning efficiency, epigenetic status, and gene expression pattern vary among cells having different origin although they may be from the same tissue.

1. Introduction

Wild buffaloes (*Bubalus arnee*) belong to the bovidae family and are found in India, Nepal, Bhutan, Cambodia, and Thailand. Since 1986, this species is listed as endangered in the International Union for Conservation of Nature Red List and is also a Schedule I animal under the Wildlife Protection Act, 1972. Today, the total wild buffalo population is less than 4000, with an estimate of less than 2500 mature animals globally [1]. The most important reason for decline in the population of wild buffalo is interbreeding with feral and domestic buffalo, hunting, and habitat loss/degradation. Diseases and parasites, transmitted by domestic livestock, and interspecific competition for food and water between wild buffalo and domestic stock are also serious threats. In central India, wild buffaloes are found only in Udanti, Indravati Tiger Reserve. Only one female

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wild buffalo is available in Udanti Wildlife Sanctuary (http://www.wti.org.in), which is kept in semicaptivity. In view of the fast dwindling population of this endangered species, there is an urgent need to increase their population.

Advanced reproductive technologies such as cryopreservation of gametes/embryos, IVF, artificial insemination, embryo transfer, and SCNT are being increasingly applied in attempts to conserve and manage endangered species. However, the lack of availability of oocytes and recipients precludes the use of traditional SCNT in exotic or endangered species. Interspecies SCNT (iSCNT) may be the only alternative available for producing embryos and offspring [2]. Birth of live offspring from iSCNT embryos followed by embryo transfer to domestic recipients has been reported in the gaur [3], mouflon [4], gray wolf [5,6], African wildcat [7], and mountain goat [8]. These studies have reported the potential of iSCNT in conservation of endangered species.

Among many others, the choice of the donor cell type for SCNT is very important because its capability to be reprogrammed by the oocyte cytoplasm is influenced by its epigenetic status. A very wide variety of donor cells of different origin and from various tissues has been used for SCNT with observed differences in the overall efficiency. These include fibroblasts from skin, ovarian cumulus cells, mammary epithelial cells, skin cells from internal organs, Sertoli cells, macrophages, oviduct epithelial cells, granulosa cells, muscle cells, neural stem cells, blood leukocytes, lymphocytes, natural killer T cells, mature B and T cells, neural and embryonic stem cells, uterine epithelial cells, olfactory cells, and so forth. (for review see [9]). We have previously reported that buffalo oocytes have the potential to reprogram somatic cells from cattle and goat up to the blastocyst stage [10]. In addition, we have recently reported successful production of iSCNT embryos produced through hand-made cloning (HMC) using recipient oocytes from domestic buffalo (*Bubalus bubalis*) and somatic cells from male wild buffalo [11]. Very recently, an interspecies cloned buffalo calf "Deepasha" was born in our laboratory (unpublished data). In this study, we compared the ability of donor cells of fibroblast and epithelial origin from wild female buffalo ear skin tissue to be reprogrammed by domestic buffalo oocytes in terms of the developmental competence and quality of iSCNT embryos produced. We also compared the global level of acetylation of histone H3 at lysine 18 (H3K18ac) and that of histone H3 trimethylated at lysine 27 (H3K27me3) in donor cells of two types and in blastocysts produced from them. In addition, the expression level of some important apoptosis- (*CASPASE3*, *CASPASE7*, and *P53*), epigenetics- (*DNMT1*, *DNMT3a*, and *HDAC1)*, and pluripotency-related genes *(OCT4*, *NANOG*, and *CDX2*) was compared in donor cells of the two types and/or in blastocysts produced from them.

2. Materials and methods

In vitro culture of somatic cells, oocytes, and embryos was done at 38.5 °C in a CO_2 incubator (5% CO_2 in air). Animal experiments were conducted after following the guidelines laid down by the Committee for the Purpose of Control and Supervision on Experiments on Animals and the Institute Animal Ethics Committee.

2.1. Isolation and culture of ear skin–derived somatic cells

Biopsy of skin tissue was taken aseptically from the lone adult female wild buffalo (*B arnee*) kept in semicaptivity at Udanti Wildlife Sanctuary, Chhattisgarh, India, with the help of an ear notcher in sterile Ca^{2+} and Mg^{2+}–containing Dulbecco's phosphate-buffered saline (DPBS) supplemented with 50-μg/mL gentamicin (DPBS+), and the tissue was airlifted to the laboratory within 24 hours at 4 °C. The cells were established as described earlier [11]. Briefly, the tissue was finely cut into 1- to 2-mm size pieces, which were cultured in 10 μL of Dulbecco's modified eagle media/F12 medium (1:1 ratio) supplemented with 20% fetal bovine serum, 0.68-mM L-glutamine, and 50 μg/mL gentamicin in a CO_2 incubator in T-25 culture flasks. After 12 hours, 3 mL of fresh medium was added to the flask to keep the attached explants submerged. The cells from the outgrowth were removed by trypsinization after they reached confluence, which usually took 5 to 7 days. For separation of cells of fibroblast and epithelial origin by differential trypsinization, the cell monolayer was washed with Ca^{2+} and Mg^{2+}–free DPBS containing 50 μg/mL gentamicin (DPBS−), incubated with 0.25% trypsin-EDTA solution at 38.5 °C for 2 minutes, and monitored closely under a microscope. When putative fibroblast cells become rounded up and began to detach from the surface, culture flasks were tapped gently and the detached cells were removed. The putative epithelial cell colonies, which remained attached tightly to the surface, were rinsed again with DPBS− and retrypsinized at 38.5 °C for 5 to 7 minutes. The separated cells were subcultured and grown in T-25 flasks till they attained confluence after which they were passaged up to 10 times. Aliquots of cells at early passages (passage 2–3) were cryopreserved in Dulbecco's modified eagle media/F12 containing 10% DMSO and 20% fetal bovine serum and were stored in liquid nitrogen for future use.

2.2. Immunocytochemical characterization of cells

Cells at passage 2 to 3 were cultured in 96-well plates (approximately 2000 cells/well) till they attained 70% to 80% confluence; after which, they were fixed for 1 hour in 4% paraformaldehyde (in DPBS+). The cells were permeabilized by treatment with 0.5% Triton X-100 for 30 minutes, blocked with 3% BSA and incubated for 1 hour with the mouse primary antibody (anticytokeratin, 1:500, SC-32329; Santa Cruz Biotechnology, CA, USA; antikeratin, 1:500, MAB1611; Millipore, MA, USA; antivimentin, 1:500; V6630; Sigma) diluted in the blocking solution and then with the secondary antibody (goat antimouse immunoglobulin G) conjugated with fluorescein isothiocyanate (FITC) for 1 hour. Positive controls used for testing the staining protocol were from each respective cell culture and were labeled with mouse antitubulin (1:500, T8328; Sigma), whereas the addition of the primary antibody was omitted in the negative controls. The cells were incubated for 10 minutes in10 μg/mL Hoechst 33342 for staining the nuclei. For detecting the fluorescence, the cells were