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Developmental competence and gene expression in preimplantation bovine embryos derived from somatic cell nuclear transfer using different donor cells

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Summary

This study compared the developmental competence of somatic cell nuclear transfer (SCNT) embryos reconstructed with different donor cells and analysed gene expression in the resulting embryos. Bovine fetal/adult ear fibroblasts and cumulus cells were used as donor cells and the developmental competence of the reconstructed embryos was monitored. The cell number and allocation in blastocysts were determined by differential staining. The Bax, E-cad, IF-tau, Hsp (heat shock protein) 70, Igf2r (insulin-like growth factor 2 receptor), DNMT (DNA methyltransferase) 1 and Mash (mammalian achaete-scute homologue) 2 genes were selected for gene expression analysis. The relative abundance (ratio to GAPDH mRNA) of gene transcripts in blastocysts was measured by semiquantitative reverse transcription-polymerase chain reaction. In experiment 1, development of SCNT preimplantation embryos and the cell numbers of inner cell masses and trophoblasts were not different among SCNT embryos derived from different cell types. In experiment 2, the relative expression of GAPDH and Hsp 70 transcripts was similar in all embryos. The expression of Bax, Igf2r and Mash2 transcripts was significantly increased in SCNT embryos reconstructed with adult fibroblasts. The E-cad transcript levels were reduced in SCNT embryos reconstructed with fetal fibroblasts. Relative abundance of DNMT1 in SCNT embryos derived from fetal fibroblasts was increased, and IF-tau expression in SCNT embryos derived from cumulus cells was increased. In conclusion, depending on the type of donor cells, preimplantation SCNT embryos displayed marked differences in gene expression. This may affect the developmental competence of SCNT embryos reconstructed with different cell types after implantation or during fetal growth *in vivo*.

Keywords: Bovine embryo, Development, Donor cells, Gene expression, SCNT

Introduction

Somatic cell nuclear transfer (SCNT) is considered to be a powerful tool to make cloned animals and transgenic animals, and to help rescue endangered animals. Since the cloned sheep 'Dolly' was produced (Wilmut *et al*., 1997), to date, live cloned offspring of sheep (Schnieke *et al*., 1997), cow (Cibelli *et al*., 1998), goat (Baguisi *et al*., 1999), pig (Onishi *et al*., 2000), mule (Woods *et al*., 2003) and horse (Galli *et al*., 2003) from fetal fibroblasts, mouse (Wakayama *et al*., 1998) and cat (Shin *et al*., 2002) from cumulus cells, and rabbit (Chense *et al*., 2002) from adult somatic cells have been born by SCNT. However, the viability of SCNT embryos and survival rate of cloned animals is still very low.

A number of studies have reported the importance of donor cell selection for improving developmental competence of SCNT embryos and production of cloned animals (Kato *et al*., 2000; Cho *et al*., 2002;

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Skrzyszowska *et al*., 2002; Lee *et al*., 2003). For example, for the production of cloned cattle a few cell types have been used for SCNT but it is not known which cell types are the most successful. Cloned calves were produced by nuclear transfer of fetal fibroblasts (Cibelli *et al*., 1998; Zakhartchenko *et al*., 1999), cumulus/oviduct cells (Kato *et al*., 1998), ear skin fibroblasts and long-term cultured cells of adult fibroblasts (Kubota *et al*., 2000). Kato *et al*. (2000) compared the efficiency in SCNT with skin, kidney, gut and muscle cells from female bovine fetuses, as well as skin, heart, liver, kidney, gut and tongue cells from male bovine fetuses. Their results demonstrated no significant differences in developmental rates of SCNT embryos derived from fetal, newborn or adult cells. Embryos derived from male fetal skin and ear cells and transferred to recipient cows resulted in the birth of one calf. Therefore, search for an ideal type of donor cells is an important step to improve efficiency of bovine SCNT and subsequent production of cloned animals.

To increase the efficiency in SCNT outcome, analysis of associated procedures such as donor cell types, activation and culture conditions has shown that preimplantation-stage embryo development is accompanied and regulated by differential expression of developmentally important genes (Zimmerman & Schultz, 1994; Schultz *et al*., 1999). Thousands of genes have to be expressed in a stage-specific manner to ensure normal embryo development, but only about 15 physiological functions and expression of 60–70 genes have been studied so far (Niemann & Wrenzycki, 2000). For example, Daniels *et al*. (2001) demonstrated the effect of different donor cell lines and nuclear transfer procedures on the expression of developmentally important genes in nuclear transfer embryos. The identification of a cohort of such genes will provide a useful tool to analyse the developmental potential of nuclear transfer embryos reconstructed using different donor cell types.

This study was performed to examine the effect of donor cells on the developmental competence of SCNT embryos and expression of developmentally important genes in order to select the ideal donor cell type in bovine SCNT. We compared the developmental rates of bovine SCNT embryos reconstructed with fetal, cumulus and adult cells. Furthermore, we analysed gene expression pattern in the resulting preimplantation embryos. The genes include *Bos taurus* apoptosis regulator box-α (Bax), E-cadherin (E-cad), interferon tau (IFN-τ), heat shock protein 70 (Hsp 70), insulin-like growth factor 2 receptor (Igf2r), DNA methyltransferase (DNMT) 1, mammalian achaete-scute homologue (Mash) 2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Materials and methods

Preparation of donor cells

Fetal fibroblasts were isolated from bovine fetuses on day 40 of gestation. The head of the fetus was removed using iris scissors, and soft tissues such as liver and intestine were discarded by scooping out with two watchmaker's forceps. After washing three times with DPBS (cat. no. 14190-144, Life Technologies, Rockville, MD), the carcass was minced with a surgical blade in a 100 mm culture dish (Becton Dickinson, Lincoln Park, NJ). For adult fibroblasts, small pieces of ear tissue biopsies from an adult Holstein cow (I.D.: Guhak) were washed three times in DPBS and minced with a surgical blade. Cumulus cells were obtained from cumulus–oocyte complexes (COCs) aspirated from large follicles of slaughterhouse ovaries and cultured up to confluence. The minced fetal and adult tissues were dissociated in Dulbecco's modified Eagle's medium (DMEM, Life Technologies) supplemented with 0.25% (w/v) trypsin and 1 mm EDTA (Life Technologies) for 1 h at 37 °C. Trypsinized cells were washed once in Ca^{2+}- and Mg^{2+}-free DPBS by centrifugation at 43 *g* for 2 min, and subsequently seeded into 100 mm plastic culture dishes.

Seeded cells were subsequently cultured for 6–8 days in DMEM supplemented with 10% (v/v) FBS (Life Technologies), 1 mM glutamine (Life Technologies), 25 mM $NaHCO_3$ (Sigma-Aldrich, St Louis, MO) and 1% (v/v) minimal essential medium (MEM) non-essential amino acid solution (Life Technologies) at 39 °C in a humidified atmosphere of 5% CO_2 and 95% air. After removal of unattached clumps of cells or explants, attached cells were further cultured until confluent, subcultured at intervals of 4–6 days by trypsinization for 5 min using 0.1% trypsin and 0.02% EDTA, allocated to three new dishes for further passaging and then stored in freezing medium in liquid nitrogen at –196 °C. The freezing medium consisted of 80% (v/v) DMEM, 10% (v/v) DMSO (Sigma-Aldrich) and 10% (v/v) fetal bovine serum (FBS). Fresh cells at passages 4 to 6 were used for SCNT. Prior to SCNT, cells were thawed, cultured for 3–4 days until 100% confluency for contact inhibition, and retrieved from the monolayer by trypsinization for 30 s.

In vitro maturation of immature oocytes

Bovine ovaries collected from a local slaughterhouse were transported to the laboratory within 2 h in a 0.9% (v/v) NaCl solution at 35 °C. The COCs were retrieved from small antral follicles 3–8 mm in diameter by aspiration with an 18 gauge hypodermic needle attached to a 10 ml syringe and washed several times in Hepes-buffered tissue culture medium (TCM)-199

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(cat. no. 11150-059; Life Technologies) supplemented with 10% (v/v) FBS, 2 mM $NaHCO_3$, 5 mg/ml bovine serum albumin (BSA; Life Technologies), and a 1% (v/v) mixture of penicillin and streptomycin (Sigma-Aldrich). The COCs with evenly granulated cytoplasm and enclosed by more than three layers of compact cumulus cells were selected. A group of 30–40 COCs were cultured for maturation in one well of a multi-well dish containing 0.5 ml of bicarbonate-buffered TCM-199 supplemented with 10% (v/v) FBS, 0.005 IU/ml FSH (Antrin, Teikoku Seiyaku, Denka, Kanagawa, Japan) and 1 μg/ml 17β-estradiol (cat. no. E-8875, Sigma-Aldrich) at 39 °C in a humidified atmosphere of 5% CO_2.

Preparation of recipient oocytes for somatic cell nuclear transfer

At 22 h of maturation culture, cumulus cells were removed by repeated pipetting in 0.1% (v/v) hyaluronidase (from bovine testis, cat. no. H-3884, Sigma-Aldrich) in Hepes-buffered Ca^{2+}-free CR2 medium (Rosenkrans *et al*., 1993) with amino acids (hCR2aa), and oocytes with a first polar body were selected. Oocytes were enucleated with a micromanipulator (Narishige, Tokyo, Japan) in hCR2aa supplemented with 10% (v/v) FBS and 5 μg/ml cytochalasin B (cat. no. C-6762, Sigma-Aldrich). Each oocyte was held with a holding micropipette (120 μm inner diameter) and the zona pellucida was partially dissected with a fine glass needle to create a slit near the first polar body. The first polar body and adjacent cytoplasm, presumably containing the metaphase II chromosomes, were extruded by squeezing with the same needle. Oocytes were then stained with 5 μg/ml bisbenzimide (Hoechst 33342, Sigma-Aldrich) for 5 min and observed under an inverted microscope equipped with epifluorescence at ×200 magnification. Oocytes still containing DNA material were excluded. The enucleated oocytes were placed in TCM-199 supplemented with 10% (v/v) FBS and used for SCNT.

Reconstruction of embryos and culture

A single cell was deposited into the perivitelline space of enucleated oocytes treated with 100 μg/ml phytohaemagglutinin (cat. no. L-9132, Sigma-Aldrich) in hCR2aa to improve the incorporation of the donor somatic cell with recipient cytoplast. The couplets were subsequently placed in a fusion medium comprising 0.26 M mannitol, 0.1 mM $MgSO_4$, 0.5 mM Hepes and 0.05% (w/v) BSA, and transferred into a cell fusion chamber with a stainless steel wire electrode (BTX 453, 3.2 mm gap; BTX, San Diego, CA) after equilibration for 3 min. Fusion was induced by two DC pulses of 1.75–1.85 kV/cm for 15 s using a BTX Electro-cell Manipulator 200. The fusion of the donor cell and the ooplast was observed 1 h after electric stimulation under a stereomicroscope. Only fused embryos were selected and cultured for 4 h in modified synthetic oviductal fluid (mSOF). The formula of mSOF was basically the same as the original formulation (Tervit *et al*., 1972) except for the concentration of 1.5 mM glucose and addition of 2% MEM essential and 1% non-essential amino acids, 8 mg/ml BSA, and 1% (v/v) mixture solution of insulin, transferrin and selenium. The osmolarity and pH of mSOF were 270–280 mOsm and 7.2 to 7.3, respectively. Chemical activation was induced by incubating embryos in mSOF containing 5 μM ionomycin (Sigma-Aldrich) for 4 min at 39 °C. Reconstructed embryos were then washed thoroughly in ionomycin-free mSOF and further incubated for 4 h in mSOF supplemented with 1.9 mM of 6-dimethylaminopurine (6-DMAP, cat. no. D-2629, Sigma-Aldrich).

After reconstruction, a group of 5 or 6 embryos was cultured in 25 μl microdrops of mSOF overlaid with mineral oil (Sigma-Aldrich) for 7 days at 39 °C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Embryo development to the 2-cell and blastocyst stages was observed at 24 and 168 h after the start of culture. The resulting blastocysts were randomly allocated for differential staining and for analysis of gene expression.

Differential staining of inner cell mass and trophectoderm cells

The numbers of blastomeres, inner cell mass (ICM) and trophectoderm (TE) cells in blastocysts and the ratio of ICM cells to total cell number per blastocyst were counted by chemically defined staining as described by Thouas *et al*. (2001). Blastocysts were incubated in 500 μl of BSA-free, Hepes-buffered TCM-199 supplemented with 1% (v/v) Triton X-100 and 100 μg/ml propidium iodide for 30 s. When the TE color visibly changed to red and shrank slightly, blastocysts were incubated at 4 °C overnight in 500 μl fixative solution consisting of 25 μg/ml bisbenzimide in absolute ethanol. The blastocysts were then treated with 99% (v/v) glycerol, mounted on a glass microscope slide in a droplet of glycerol solution. The cell numbers in each category were counted using epifluorescence microscopy. The ICM cell nuclei labelled with bisbenzimide appeared blue and TE nuclei labelled with both bisbenzimide and propidium iodide appeared pink.

Determination of the relative abundance of gene transcripts in bovine preimplantation embryos derived from SCNT

Six blastocysts were washed in three changes of PBS and transferred into 0.2 ml of 4 M guanidium

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Table 1 Details of primers used for RT-PCR

Gene	Primer sequences	Annealing temperature (°C)	Cycle number	Fragment size (bp)
Bax	5′-TGCAGAGGATGATCGCAGCTGTG 3′-CCAATGTCCAGCCCATCATGGTC	60	30	198
E-cad	5′-AGGCCCCTGTGCAGCTGGCTCAAATCAA 3′-AGGCCCCTGTGCAGCTGGCTCAAATCAA	60	30	332
IF-tau	5′-GCTATCTCTGTGCTCCATGAGATG 3′-AGTGAGTTCAGATCTCCACCCATC	55	30	359
Hsp 70	5′-AAGGTGCTGGACAAGTGCCAGGAGGTGA 3′-ACTTGGAAGTAAACAGAAACGGGTGAAAA	55	30	488
Igf2r	5′-CGCCTACAGCGAGAAGGGGTTAGTC 3′-AGAAAAGCGTGCACGTGCGCTTGTC	60	50	293
DNMT1	5′-CGCATGGGCTACCAGTGCACCTT 3′-GGGCTCCCCGTTGTATGAAATCT	55	50	312
Mash2	5′-CGCTGCGCTCGGCGGTGGAGTA 3′-GGGACCCGGGCTCCGAGCTGTG	65	50	210
GAPDH	5′-CATCACCATCTTCCAGGAGCGAGA 3′-CCTGCTTCACCACCTTCTTGATGT	55	30	573

isothiocyanate lysis solution containing 1% β-mercaptoethanol. Total RNA was extracted by thiocyanate extraction and dissolved in 10 µl RNase-free water as described by Szafranska *et al*. (1995) and subjected to reverse transcription-polymerase chain reaction (RT-PCR). Reverse transcription was carried out at 37 °C for 60 min. Individual RT reactions (15 µl each) consisted of 5 mM $MgCl_2$, 1× RT buffer, 2.5 µM oligo(dT), 1 mM dNTP and 50 IU murine leukaemia virus reverse transcriptase (Amersham Pharmacia Biotechnologies, Oakville, ON, Canada). Primer sequences, annealing temperature and the approximate sizes of the amplified fragments are listed in Table 1. All primers (Bax, E-cad, IF-tau, Hsp 70, Igf2r, DNMT 1, Mash 2 and GAPDH gene) were selected based on the bovine sequence of the respective genes available in the gene database at the National Center for Biotechnology Information. The cDNA (1 µl) was amplified in a 50 µl PCR reaction containing 1.25 units hot start *Taq* polymerase (Qiagen, Hilden, Germany) and its buffer, 1.5 mM $MgCl_2$, 2 mM dNTP and 25 pmol specific primers. The PCR amplification was carried out for one cycle with denaturing at 95 °C for 15 min, and 35 subsequent cycles with denaturing at 95 °C for 30 s, annealing for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min.

Ten microliters of PCR products were fractionated on a 1.5% agarose gel, and stained with ethidium bromide. For each pair of gene-specific primers, semilog plots of the fragment intensity as a function of cycle number were used to determine the range of cycle number over which linear amplification occurred, and the number of PCR cycles was kept within this range. The expression level for each gene was determined by densitometric analysis using Gel Doc software (Bio-Rad, Hercules, CA). Relative expression levels of each gene were represented as the ratio of each gene to GAPDH gene.

Statistical analysis

Data were analysed using a Statistical Analysis System (SAS) program. Random distribution of SCNT embryos was made in each experimental group and experiments were replicated at least seven times. Interaction analysis among experimental parameters was first performed. As no interactions were found, the data were subjected to analysis of variance (ANOVA) and a protected least significant different (LSD) test using general linear models to determine differences among experimental groups. When a significant model effect was found in each experimental parameter, data were compared by the least squares method. Statistical significance was accepted when the p value was less than 0.05.

Results

Evaluation of the development and cell numbers of SCNT preimplantation embryo derived from different donor cells

As shown in Table 2, developmental competence of SCNT preimplantation embryos reconstructed with the three different donor cell types were not significantly different. Blastocyst formation rates were 23.9%, 22.7% and 23.8% for SCNT with bovine fetal fibroblasts, adult ear fibroblasts and cumulus cells, respectively. The resulting blastocysts were differentially stained and had an average of 118.3 ± 13.0, 115.0 ± 19.5 and

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Table 2 Effect of three different donor cell types on preimplantation development of bovine somatic cell nuclear transfer (SCNT) embryos

No. of embryos development	Type of donor cell		
	Bovine fetal fibroblast	Adult ear fibroblast	Cumulus cells
Couplets	264	284	251
Fused (%)	201 (76.1)	225 (79.2)	202 (80.5)
Cleaved (%)	130 (64.7)	146 (64.9)	124 (61.4)
Blastocyst (%)	48 (23.9)	51 (22.7)	48 (23.8)

Table 3 Mean cell number of inner cell mass (ICM) and trophectoderm (TE) in bovine somatic cell nuclear transfer (SCNT) embryos derived from three different donor cell types

Distribution of cells in blastocyst (n = 20)	Type of donor cell		
	Bovine fetal fibroblast	Adult ear fibroblast	Cumulus cells
ICM	39.5 ± 10.7	34.8 ± 3.5	35.1 ± 7.0
TE	78.75 ± 8.9	80.3 ± 18.3	79.6 ± 22.1
Total	118.3 ± 13.0	115.0 ± 19.5	114.7 ± 20.8

The data represent mean ± SEM of cell counts on 20 blastocysts at day 8.

115.0 ± 19.5 cells, of which 39.5 ± 10.7, 34.8 ± 3.5 and 35.1 ± 7.0 were allocated to the ICM, respectively, in blastocysts derived from the three nuclear donor cell types (Table 3). No significant difference was detected between the number of ICM and TE cells from SCNT blastocysts derived from the different donor cells.

Relative abundance of mRNA transcripts expression

Representative gel photographs of a semiquantitative RT-PCR assay of the analysed gene transcripts in day 8 blastocysts are shown in Fig. 1. The expression of Bax, Igf2r and Mash2 transcripts was significantly increased in SCNT embryos reconstructed with adult fibroblasts. The E-cad transcript levels were reduced in SCNT embryos derived from fetal fibroblasts. The relative abundance of DNMT1 in SCNT embryos derived from fetal fibroblasts was increased, and IF-tau expression in SCNT embryos derived from cumulus cells was also increased.

Discussion

Cloned offspring have been produced by SCNT in several species using many kinds of somatic cells as nuclear donors. Fetal or adult fibroblasts and cumulus cells are known to be suitable donor cells. Problems with DNA methylation, acetylation, imprinting and reprogramming cause the low efficiency of successful cloning. In order to understand the cloning process, many researchers have recently suggested that gene expression related to methylation, acetylation and imprinting can be a good criterion for evaluating the viability of embryos (Reik *et al*., 2001; Rideout *et al*., 2001). In this study, we investigated the efficiency of bovine SCNT using different donor cells with respect to the pattern of gene expression. Development rates up to the blastocyst stage were similar in all groups (Table 2). Early fetal fibroblasts were mostly used as nuclear donor cells in producing the first cloned animals. Previously, our laboratory reported that in porcine SCNT, using fetal fibroblasts significantly increased development to the blastocyst stage (Lee *et al*., 2003). Kato *et al*. (2000) reported that the percentages of blastocysts that developed from SCNT with each of the donor cell types were not significantly different, except for those at the extremes of the range, such as female fetal muscle cells compared with male adult liver cells.

In general, the cell numbers of ICM and TE reflect the viability of blastocysts. ICM differentiates into all the fetal organs while TE together with uterine epithelium forms the placenta. It has been suggested that the proportion of ICM cells in blastocysts is crucial for post-implantation development (Willadsen & Polge, 1981; Iwasaki *et al*., 1990) and that an increase in total blastocyst cell numbers is correlated with improved embryo viability (van Soom *et al*., 1997). In the present study, the cell numbers of ICM and TE in preimplantation stage embryos derived from SCNT with different donor cells were not significantly different (Table 2). Previously, our laboratory reported successful births of cloned offspring derived from SCNT using various donor cells such as cumulus, adult ear fibroblasts, oviduct and uterine cells (Cho *et al*., 2002). We believe that it is impossible to identify the most appropriate donor cells by assessing the cell numbers of ICM and TE because a variety of donor cells (Cho *et al*., 2002) supported the birth of cloned cattle derived from SCNT.

For further study of preimplantation stage embryos derived from SCNT, we analysed the pattern of mRNA expression. GAPDH, selected as a control, showed a similar pattern to beta-actin and tubulin in all SCNT preimplantation embryos. Hsp 70, which protects cells against adverse effects of stress (Welch *et al*., 1987; Hendrey & Kola, 1991) and functions in the absence of stress as a molecular chaperone (Ellis & van der Vies, 1991; Becker & Craig, 1994), similarly showed relatively abundant gene expression in all embryos.

Bax, known as a pro-apoptotic of the bcl-2 family, is involved in programmed cell death. Apoptosis is part of the normal developmental programme of embryos

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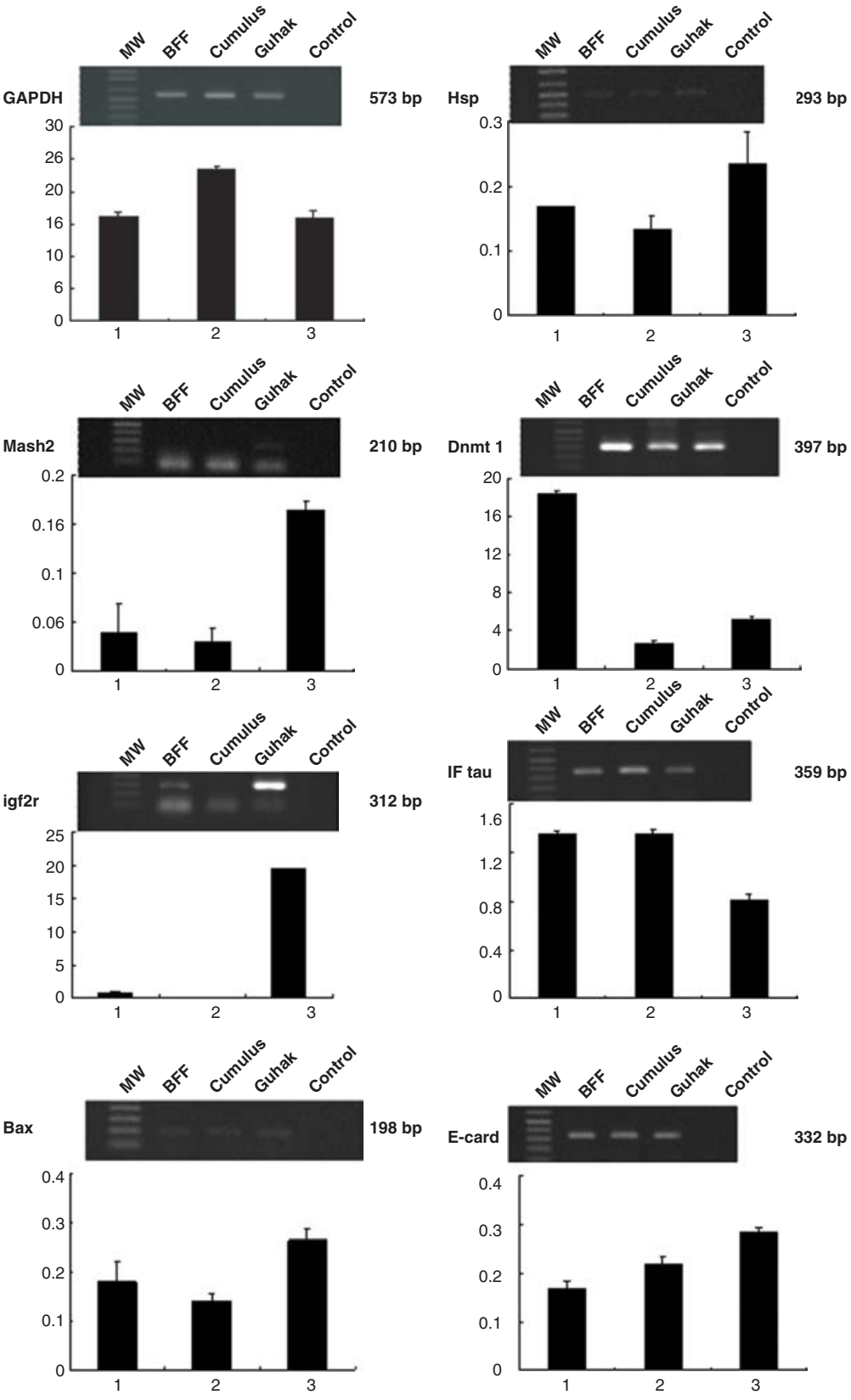

Figure 1 Relative mRNA abundance in SCNT preimplantation embryos derived from three different donor cell types. A day 8 blastocyst was chosen for analysing mRNA transcripts for each gene. BFF, bovine fetal fibroblast; Guhak, adult ear fibroblast. 1, bovine fetal fibroblast; 2, cumulus cells; 3, adult ear fibroblast.

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(Pierce *et al*., 1989; Hardy, 1997) and is developmentally regulated (Matwee *et al*., 2000). However, the percentage of apoptotic cells is significantly higher in blastocysts produced *in vitro* compared with blastocysts *in vivo* (Jurisicova *et al*., 1998). More apoptotic blastomeres were observed in SCNT preimplantation embryos derived from long-term cultured donor cells (Jang *et al*., 2004). The expression of bax in all SCNT preimplantation embryos was significantly increased in SCNT embryos reconstructed with adult fibroblasts. We suggest that the increased expression of bax in these embryos is due to the relationship between apoptosis and ageing.

The expression of Igf2r and Mash transcripts was significantly increased in SCNT embryos reconstructed with adult fibroblasts. Igf2 and Igf2r are imprinted genes that play important roles in preimplantation development and Igf2r is the receptor corresponding to the signal pathway for growth factor regulation of fetal growth. Recently, it was shown that levels of Igf2 mRNA were significantly elevated in bovine fetuses originating from *in vitro* produced embryos compared with their *in vivo* counterparts (Blondin *et al*., 2000). Overexpression of Igf2 caused abnormal growth such as 'large offspring syndrome'. The biological function of imprinting in mammals is not yet understood but it may act to regulate the supply of nutrition to the embryo in order to maintain a balance between fetal demands and maternal resources. Higher expression of Igf2r in SCNT embryos our study may mediate the effect of Igf2 and lead to production of overweight offspring. Mash, a member of the large family of basic helix-loop-helix proteins which includes many genes involved in cell type specification, is expressed in diploid trophoblast and plays a critical role in the specification of postimplantation spongiotrophoblast for normal development of the placenta (Guillemot *et al*., 1995). Overexpression of the Mash gene, like Igf2r, in SCNT embryos with reconstructed adult fibroblasts may lead to abnormality in the placenta which guarantees growth and survival of the developing embryo in the uterine environment of the mother.

E-cad contributes to the maintenance of tissue integrity by enhancing cell–cell adhesion and is also involved in cell–cell communication. Downregulation of E-cad contributes to the motility and invasiveness of implantation-site intermediate trophoblastic cells during implantation (Shih *et al*., 2002). Our results may suggest that the reduction of the E-cad gene in SCNT embryos derived from fetal fibroblasts may be a beneficial effect for implantation.

Among the three different donor cells, transcript levels of DNMT were increased in SCNT preimplantation embryos derived from fetal fibroblasts. DNMT1 is the most extensively studied and abundant DNA methyltransferase and is thought to be responsible for copying methylation patterns following DNA synthesis (Robertson *et al*., 1999). Abnormally high DNMT transcript levels could be correlated with methylation errors even in *de novo* methylation that would then be copied after cell division (Jones, 1996). If persistent, this could lead to epigenetic changes suspected to be associated with the abnormalities seen in *in vitro* produced and SCNT offspring (Young *et al*., 1998; Niemann & Wrenzycki, 2000).

IF-tau expression was increased in SCNT embryos derived from cumulus cells compared with other SCNT embryos derived from fetal or adult fibroblasts. IF-tau is secreted by trophectoderm and is related to the implantation of bovine blastocysts. This gene product inhibits Cox to assist implantation of the embryo. Inhibition and deficiency of Cox, which generates prostaglandin via the cyclooxygenase pathway, cause abnormalities in ovulation, fertilization, implantation and decidualization (Langenbach *et al*., 1995; Lim *et al*., 1997).

In conclusion, the present results demonstrate that SCNT with three different donor cells can alter the expression patterns of developmentally important genes in preimplantation embryos. From our results, we suggest that even though donor cells from various tissues and organs can support development to the blastocyst stage, differences in donor cell nucleus behaviour in recipient oocytes, such as reprogramming, imprinting and methylation, may play a crucial role in fetal growth *in vivo* and in producing healthy cloned offspring.

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