Expression of pluripotency markers in Arbas Cashmere goat hair follicle stem cells

Nimantana He[1] · Zhenguo Dong[1] · Bing Zhu[1] · Mingtu Nuo[1] · Shorgan Bou[1] ·
Dongjun Liu[1]

Abstract In our previous work, we found that the Inner Mongolia Arbas Cashmere goat hair follicle stem cells (gHFSCs) can be successfully differentiated into adipocyte, chondrocyte, and osteocyte lineages. In this study, we further examined the expression of the pluripotency and stemness markers Oct4, Nanog, Sox2, AKP, and TERT in gHFSCs by immunocytochemistry, flow cytometry, real-time PCR, and Western blot. Immunofluorescent staining showed that the gHFSCs were positive for all five markers. Fluorescence-activated cell sorting (FACS) further analyzed the positive expression of Oct4, Nanog, and Sox2 in the gHFSCs. Compared with Arbas Cashmere goat adipose-derived stem cells (gADSCs) at the mRNA expression level, Oct4 was relatively highly expressed in gHFSCs, 41.36 times of the gADSCs, and Nanog was 5.61, AKP was 2.74, and TERT was 2.10 times, respectively ($p < 0.01$). Western blot indicated that all markers are expressed at the protein level in the gHFSCs. When compared with gADSCs, using α-tubulin as a reference protein, gray intensity analysis showed that the expression of Oct4, Nanog, AKP, and TERT were, respectively, 5.94, 10.78, 1.33, and 1.39 times of gADSCs. Additionally, mRNA and protein expression of Sox2 were detected in the gHFSCs but not in the gADSCs. The protein expression pattern of these markers was consistent with the mRNA results.

✉ Dongjun Liu
  nmliudongjun@sina.com

1 Key Laboratory of Mammalian Reproductive Biology and Biotechnology Ministry of Education, Inner Mongolia University, Hohhot 010070, Inner Mongolia, China

Introduction

Stem cells can be divided into embryonic stem cells and adult stem cells according to the source. Embryonic stem cells, known as totipotent cells, can theoretically differentiate into all cell types both in vivo and in vitro. Embryonic stem cell research has been greatly restricted because of difficulty in obtaining cells, immunological rejection, and ethical and moral issues. Adult stem cells are undifferentiated cells that exist in the differentiated tissue of a mature individual. They have the ability of self-renewal and multipotent differentiation and are easy to obtain, and autotransplantation can be performed. With these advantages, adult stem cells have gradually become the current direction of cell therapy (Trounson et al. 2011).

The skin, which covers on the surface of the entire body, has a variety of functions, including thermoregulation, physical protection, sensory activity, and social interactions. Furthermore, it undergoes continuous self-renewal throughout the lifetime and also has an extensive ability to repair wounds. For these reasons, a stem cell population has long been suspected to exist within the epithelial compartment of the skin (Nowak and Fuchs 2009). Hair follicle stem cells (HFSCs) are required to generate, maintain, and renew the continuously cycling hair follicle (HF); supply cells that produce the keratinized hair shaft; and aid in the re-epithelialization of injured skin. HFSCs localize to the basal, outermost ORS layer of the distal HF epithelium at the proximal end of the isthmus, known as the "bulge" (Jimenez et al. 2010; Ohyama et al. 2006). Ever since their identification in mice (Cotsarelis et al. 1990), the biology of HFSCs has

become a very fertile and exciting frontier not only in the HF field but also in general epithelial biology (Purba et al. 2014). Previous studies showed that human and mouse HFSCs cannot only differentiate into hair follicles but also can differentiate into nerve cells, glial cells, smooth muscle cells, and epithelial cells (Taylor et al. 2000; Amoh et al. 2005; Yu et al. 2006; Liu et al. 2011; Yashiro et al. 2015). Stem cell biological research extends to basic medical and clinical therapy and is penetrating into every field of life science and medicine. In addition, stem cell techniques can be applied in animal husbandry to improve the quality and production of domestic animals.

Pluripotency describes the developmental competence of cells that display a specialized differentiation capability: the ability to give rise, through differentiation, to all cell types of the embryo and adult. In this study, we detected the expression of a group of pluripotency markers which are essential for the stem cell pluripotent state and self-renewal, such as Oct4, Nanog, Sox2, AKP, and TERT in the gHFSCs, and furthermore, explore its pluripotency and stemness.

Materials and Methods

Cells We used gHFSCs and gADSCs that were isolated and identified in the Key Laboratory of Mammalian Reproductive Biology and Biotechnology Ministry of Education, Inner Mongolia University, Hohhot, China.

Reagents The following reagents were used: DMEM/F-12 (SH30023, Hyclone, Logan, UT), PBS (SH30028, Hyclone), penicillin–streptomycin mixture (SV30010, Hyclone), fetal bovine serum (FBS; TBD31HB, tbd, Tianjin, China), EGF (E1257, Sigma, St. Louis, MO), insulin (I0516, Sigma), hydrocortisone (H0888, Sigma), paraformaldehyde (P6148, Sigma), TritonX-100 (9002-93-1, Sigma), DAPI (D8417, Sigma), and RIPA lysis buffer (2089131, Millipore, Darmstadt, Germany). Pierce® BCA Protein Assay Kit

(NC13227CH, Thermo Scientific, Waltham, MA) was used. Primary antibodies against Oct4 (ab18976), Nanog (ab80892), Sox2 (ab137385), AKP (ab97384), TERT (ab183105), and α-tubulin (ab176560) and secondary antibodies goat anti-rabbit IgG AlexaFluor®488 (ab150077) and horseradish peroxidase-linked goat anti-rabbit IgG (ab6721) were all purchased from Abcam (Cambridge, MA). RNAiso Plus (RR9109), PrimeScript™ RT reagent kit (RR047A), and SYBR® Premix Ex Taq (RR820A) were all purchased from Takara (Dalian, China).

Cell Culture The gHFSCs culture medium was DMEM/F12 supplemented with 2% FBS, 1% penicillin–streptomycin mixture, 10 ng/mL EGF, 10 ng/mL insulin, and 0.4 μg/mL hydrocortisone. The gADSC culture medium was DMEM/F12 supplemented with 20% FBS. Cells were cultured at 37°C with 5% CO_2, and medium was changed every 3 d. Cell morphology and growth were observed microscopically.

Immunocytochemistry The gHFSCs grown on 24-well coverslips were fixed with 4% paraformaldehyde at room temperature for 15 min. Cells were permeabilized with 0.1% Triton X-100 in PBS for 15 min. After washing (three times), the specimens were blocked in 10% goat serum-PBS for 1 h at room temperature. The cells were incubated with the primary antibodies for 1 h at 37°C. Primary antibodies against Oct4, Nanog, Sox2, AKP, and TERT were diluted at 1:400. The negative control was incubated with 10% goat serum-PBS instead of primary antibody. After washing (three times), cells were incubated with FITC-conjugated goat anti-rabbit secondary antibody (1:500) in the dark for 45 min at room temperature. After washing (three times), the nuclei were counterstained with DAPI (1:1000) for 5 min at room temperature in the dark. At least three replicates were performed for each sample. The cells were visualized using a confocal microscope (A1 confocal, Nikon).

Table 1 Sequences of the primers

Gene name	NCBI accession	Sequence		Product
Oct4	XM_013977063.1	Forward	5′-GCCAAGCTCCTAAAGCAGAAGA-3′	122 bp
		Reverse	5′-AAAGCCTCAAAACGGCAGATAG-3′	
Nanog	NM_001285576.1	Forward	5′-GTCTCTCCTCTTCCTTCCTCCA-3′	116 bp
		Reverse	5′-TCTTCCTTCTCTGTGCTCTCCTC-3′	
Sox2	NM_001285672.1	Forward	5′-CATGATGGAGACGGAACTGG-3′	115 bp
		Reverse	5′-CGGGCTGTTCTTCTGGTTG-3′	
AKP	XM_013974604.1	Forward	5′-ATGGTCACCATGAAGGCAAAG-3′	125 bp
		Reverse	5′-ATGGTCTGCAGTGGCAAGGA-3′	
TERT	XM_013972889.1	Forward	5′-GTGCTGAACTACGAGCGAGC-3′	147 bp
		Reverse	5′-GTCCGCCTTGACGAAGTAGAG-3′	
GAPDH	XM_005680968.2	Forward	5′-TTGTGATGGGCGTGAACC-3′	127 bp
		Reverse	5′-CCCTCCACGATGCCAAA-3′	

Figure 1 Cell morphology of gHFSCs and gADSCs. (A) Cultured gHFSCs showed a typical morphology of epithelial cells and a cobblestone appearance and are closely aligned with high refractive index, small cell size, centralized, round, and large nuclei. (B) Cultured gADSCs showed a typical fibroblast-like morphology with short or long spindles. *Scale bar* 100 μm.
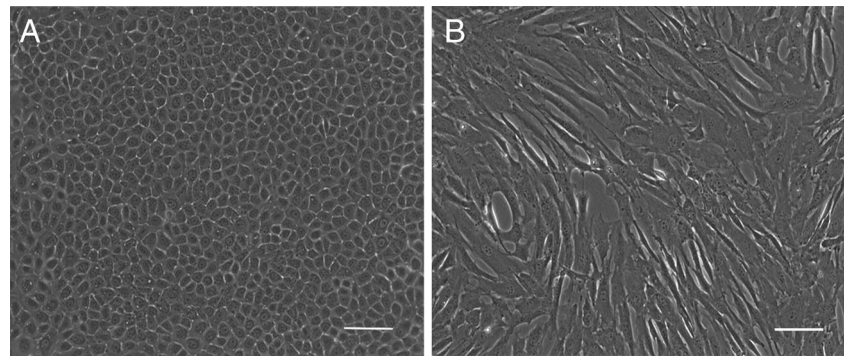
FACS After being harvested, gHFSCs were fixed in 4% paraformaldehyde for 40 min and permeabilized with 0.2% Triton X-100 in 10% goat serum for 10 min on ice then incubated with unconjugated primary antibodies for 1 h and FITC-conjugated secondary antibody for 40 min on ice sequentially. Flow cytometry was performed by BD FACSDiva 7.0 and analyzed by FlowJo.

Real-Time PCR Oct4, Nanog, Sox2, AKP, and TERT mRNA expression levels in gHFSCs and gADSCs were measured using real-time quantitative PCR. All primer sequences were determined using established GenBank sequences, which are listed in Table 1. The total RNA of the gHFSCs and gADSCs was extracted using RNAiso Plus and then reverse transcribed into cDNA with the PrimeScript™ RT reagent kit according to the manufacturer's instructions. The real-time PCR reaction system comprised 2× SYBR Green Mix (10 μL), primer mix (1 μL), template (1 μL), and ddH$_2$O (8 μL). The Q-PCR parameters were as follows: 95°C for 30 s followed by 40 cycles of 95°C for 30 s and 60°C for 30 s. To determine if there were multiple PCR amplicons, melting curves were constructed by heating final amplification reactions from 60 to 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s in single degree steps. The relative mRNA expression level of each gene from triplicate experiments was calculated using the $2^{-\Delta\Delta Ct}$ method (Schefe et al. 2006).
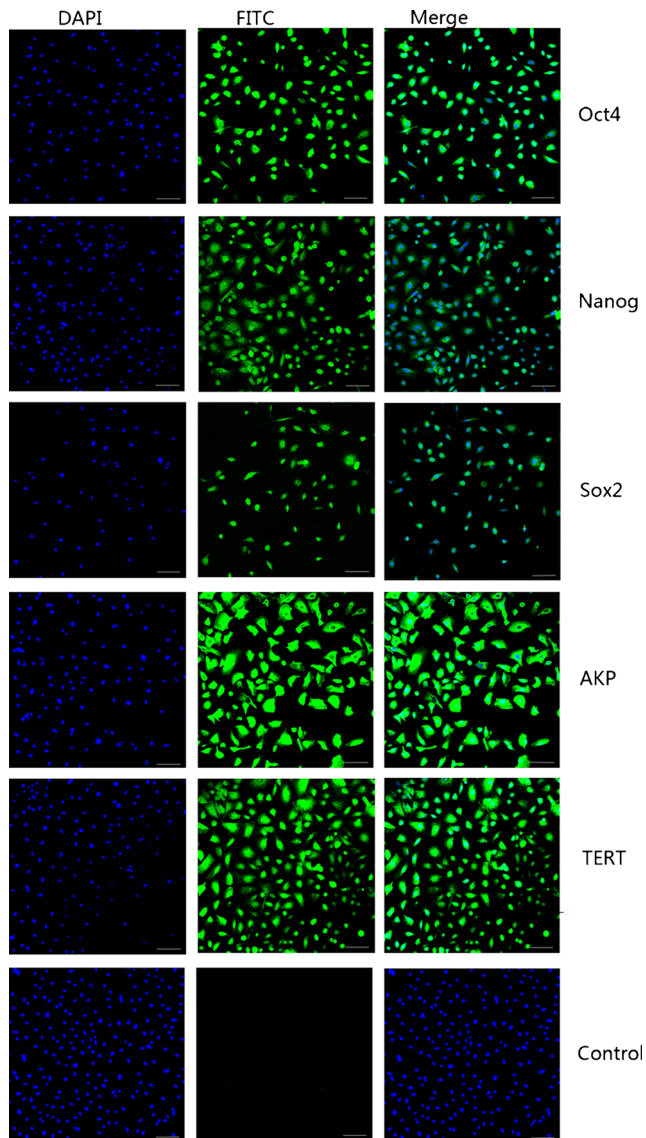

Figure 2 Immunocytochemistry staining of pluripotency markers. Immunocytochemistry staining showed positive expression of Oct4, Nanog, Sox2, AKP, and TERT in gHFSCs. Cells were incubated with primary antibodies (1:400) for 1 h at 37°C. The negative control was incubated 10% goat serum-PBS instead of primary antibody. The nuclei were counterstained with DAPI (1:1000). *Scale bar* 100 μm.

Western Blot Total cellular extracts of gADSCs and gHFSCs were obtained for the Western blot analyses by RIPA lysis buffer. Protein concentrations of the cell lysates were determined using the BCA Protein Quantitation Kit. Aliquots of cell lysates containing 15 μg of proteins were electrophoretically separated by 12% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked with TBST buffer (10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.05% Tween 20) containing 5% skimmed milk and then incubated with primary antibodies against Oct4, Nanog, Sox2, AKP, and TERT at 4°C overnight. This was followed by the addition of horseradish peroxidase-linked anti-rabbit IgG and enhanced chemiluminescence visualization of the protein bands. The intensity of each band was analyzed using ImageJ software.
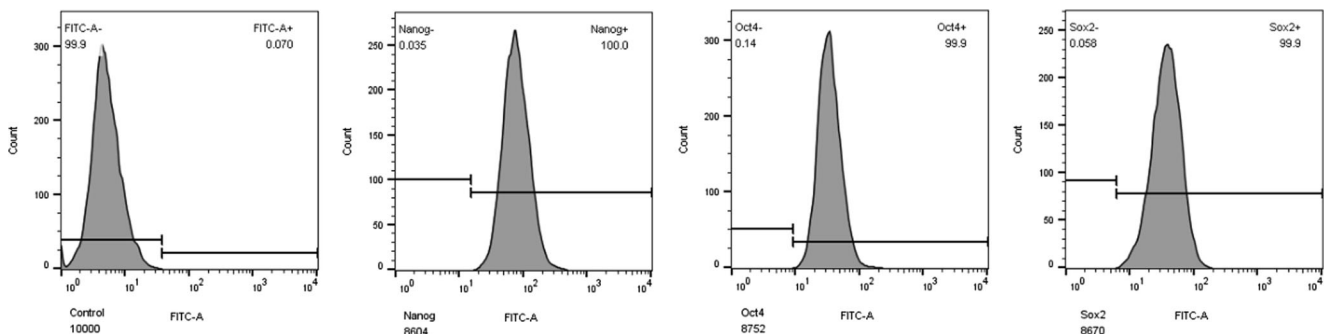
Figure 3 FACS of Oct4, Nanog, and Sox2 in the gHFSCs. Fluorescence-activated cell sorting (FACS) analysis showed positive expression of Oct4, Nanog, and Sox2 in the gHFSCs. Control was incubated with FITC-conjugated secondary antibody only. *Vertical axis*: cell number, *horizontal axis*: channel number.

Statistical Analysis All of the data were presented as a mean and standard error of the mean and analyzed using SPSS 19.0. A p value of less than 0.05 (p 0.05) was considered statistically significant. Each experiment was repeated at least three times.

Results

Cell Morphology of gHFSCs and gADSCs Cultured gADSCs showed a typical fibroblast-like morphology of short or long spindles. The gHFSCs showed a typical morphology of epithelial cells, such as a cobblestone and nest appearance, stereoscopic impression, high refractive index, small cell size, and centralized, round, and large nuclei (Fig. 1).

Immunofluorescent Staining for Pluripotency and Stemness Biomarkers Immunohistochemistry staining of
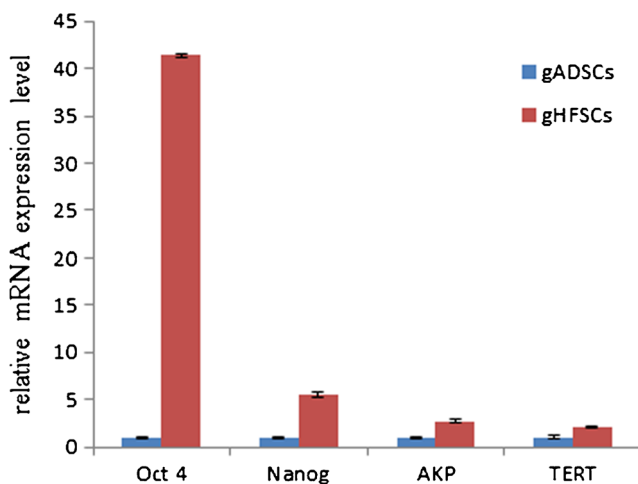

Figure 4 mRNA expression of Oct4, Nanog, Sox2, AKP, and TERT. Relative mRNA expression was tested by real-time PCR and calculated using the $2^{-\Delta\Delta Ct}$ method. All five markers were expressed in gHFSCs, and Sox2 was not detected in gADSCs. The relative expression of Oct4, Nanog, AKP, and TERT in the gHFSCs was significantly higher than their expression in gADSCs ($p < 0.01$).

Oct4, Nanog, Sox2, AKP, and TERT showed that gHFSCs were positive for all five markers (Fig. 2). Oct4, Nanog, and Sox2 were chiefly expressed in the nucleus.

FACS of Oct4, Nanog, and Sox2 in gHFSCs The expression of the three core pluripotency markers, Oct4, Nanog, and Sox2, were all detected in the gHFSCs by flow cytometry analysis (Fig. 3), and positive expression rates were equal to or greater than 99.9%. These results indicated that further investigation of these markers was feasible.

Differential Expression of Oct4, Nanog, Sox2, AKP, and TERT at the mRNA Level in gHFSCs At the mRNA level, Oct4, Nanog, Sox2, AKP, and TERT were all detected in the gHFSCs; Oct4, Nanog, AKP, and TERT were detected in gADSCs; and Sox2 was not detected in gADSCs. When compared with gADSCs, using GAPDH as the reference gene, Oct4 was relatively highly expressed in gHFSCs, that is, 41.36 times of its expression in the gADSCs, and Nanog was 5.61 times, AKP was 2.74 times, and TERT was 2.10 times, respectively ($p < 0.01$, Fig. 4). Sox2 expression in gHFSCs and gADSCs is shown in Table 2. The mRNA expression of Oct4, Nanog, Sox2, AKP, and TERT in gHFSCs was all higher than those in gADSCs.

Differential Expression of Oct4, Nanog, Sox2, AKP, and TERT Proteins in gHFSCs We detected protein expression of Oct4, Nanog, Sox2, AKP, and TERT by Western blot. All five markers were expressed in gHFSCs, and naturally, Sox2 was not detected in gADSCs (Fig. 5A). When compared with gADSCs, with α-tubulin as reference protein, gray intensity analysis showed the expression of Oct4, Nanog, AKP, and TERT in the gHFSCs were, respectively, 5.94, 10.78, 1.33, and 1.39 times of those in gADSCs (Fig. 5B). Protein expression of Oct4, Nanog, Sox2, AKP, and TERT was all higher than those in gADSCs, and the protein expression pattern of these markers was consistent with the mRNA results.

Table 2 Sox2 expression in gHFSCs and gADSCs at mRNA level

	GAPDH					Sox2					Δc_t
	c_{t1}	c_{t2}	c_{t3}	Av	StDev	c_{t1}	c_{t2}	c_{t3}	Av.	StDev	
gHFSCs	12.09	12.17	11.93	12.06	0.12	29.76	30.01	29.97	29.91	0.13	$17.85 \pm 0.03^*$
gADSCs	11.93	11.86	11.98	11.92	0.06	No c_t	No c_t	No c_t	–	–	–

Av average, *StDev* standard deviation

*Compared with GAPDH. p 0.05

Discussions

Gene expression programs guide developmental decisions and underlie cell identity during all stages of development. Several transcription factors and chromatin regulators have been identified as being integral to the establishment and/or maintenance of pluripotency, simultaneously regulating the expression of genes within pluripotent cells and acting as gene targets of these same processes. The core of the transcriptional circuitry contains "elite" factors Oct4, Sox2, and Nanog, which form autoregulatory loops and control genes that help to maintain the pluripotent state and contribute to the repression of key lineage genes. A number of additional factors are believed to endorse pluripotency by maintaining appropriate levels of the elite factors. All factors in the circuitry are highly interconnected, mutually reinforcing, and extensively redundant, which confers robustness and flexibility to the system (Chen et al. 2008; Young 2011).

The POU homeodomain transcription factor Oct4 (also known as Pou5f1) is expressed in all pluripotent cells of the mammal and is downregulated upon formation of extraembryonic and somatic lineages. In vitro research has suggested an absolute requirement for Oct4 in the establishment of the pluripotent lineage, but it is not sufficient for the maintenance of pluripotency (Niwa et al. 2002). The variant homeodomain transcription factor Nanog is expressed throughout the pluripotent cells of the inner cell mass (ICM) but is downregulated in extraembryonic lineages and pluripotent cells of the peri-implantation embryo. The Sox2 (SRY-related HMG box) gene is expressed in the ICM, early primitive ectoderm, anterior primitive ectoderm, germ cells, and multipotent extraembryonic ectoderm cells. Sox2 was not detected in adult stem cells in the recent study (Ren et al. 2014). Sox2$^{-/-}$ embryos arrest at a similar time as Oct4$^{-/-}$ and Nanog$^{-/-}$ embryos and blastocyst-like structures are formed, but developmental arrest, characterized by a lack of primitive ectoderm, occurs around the time of implantation (Brett et al. 2006). However, recent evidence suggests that Nanog may function to stabilize the pluripotent state rather than being essential for maintaining pluripotency of embryonic stem (ES) cells (Chambers et al. 2007). Oct4 can heterodimerize with Sox2 in ES cells, and Sox2, in turn, contributes to pluripotency, at least in part, by regulating Oct4 levels (Masui et al. 2007). Oct4 is rapidly and apparently completely silenced during early cellular differentiation. The key roles played by Oct4, Sox2, and Nanog during early development, along with their unique expression pattern, make it likely that these regulators are central to the transcriptional regulatory hierarchy that specifies embryonic stem cell and pluripotent stem cell identity (Jaenisch and Young 2008).

The telomerase reverse transcriptase (TERT) component binds an RNA component that aligns telomerase to the
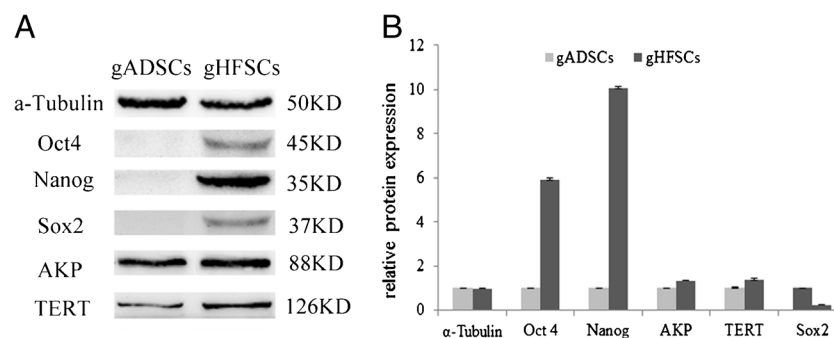

Figure 5 Protein expression of Oct4, Nanog, Sox2, AKP, and TERT. Protein expression was examined by Western blot and analyzed using ImageJ. All five markers were expressed in gHFSCs and Sox2 was not detected in gADSCs (*A*). When compared with gADSCs, using α-tubulin as reference protein, the expression of Oct4, Nanog, AKP, and TERT in the gHFSCs was significantly higher (*B*) than their expression in gADSCs (*p* < 0.01).

chromosomal ends and acts as a template for the addition of telomeric DNA (de Lange 2009). High telomerase activity is characteristic of high renewal capacity in cells and tissues. Telomerase-deficient mice with critically short or uncapped telomeres exhibit tissue atrophy, stem cell depletion, organ system failure, and impaired tissue (Shay and Wright 2010). These observations support the hypothesis that telomere length and telomerase activity are determinants for tissue homeostasis and regeneration (Podlevsky and Chen 2012).

In the field of stem cell biology, tissue nonspecific alkaline phosphatase (TNAP) is the focus in all alkaline phosphatase (AKP) isoenzymes. A high level of AKP and high AKP activity are traditional markers of pluripotent ES cells and a widely accepted marker of pluripotent stem cells. The expression of AKP is quickly upregulated directly after the transfection of the four genes (Oct4, Sox2, Klf4, C-myc) during the process of reprogramming somatic cells into iPS cells (O'Connor et al. 2008). This corresponds with the conception of the direct regulation of AKP expression by Oct4 and Sox2 (Yamanaka and Takahashi 2006). In silico analysis identified binding sites for Oct4, Nanog, Sox2, and other factors associated with pluripotency such as Tcf3, Sa4b, and FoxD3 in promoters of AKP (Štefková et al. 2015).

In our study, three core transcription factors, Oct4, Nanog, and Sox2, were found to have a positive expression rate of greater than 99.9% by flow cytometry. In addition, Oct4, Nanog, Sox2, TERT, and AKP were all detected by immunofluorescent staining, Q-PCR, and Western blot in gHFSCs. With gADSCs as control, at the mRNA expression level, Oct4 was relatively high in gHFSCs and Nanog and AKP and TERT were at a moderate level. At the protein level, the expression of Nanog in the gHFSCs was high, and it was medium for Oct4, AKP, and TERT. Moreover, Sox2 expression at the mRNA and protein level was all detected in the gHFSCs, but not in gADSCs. That is, these pluripotency biomarkers Oct4, Nanog, Sox2, AKP, and TERT showed significantly higher expression in the gHFSCs (p 0.01) both at the mRNA and protein level, and their RNA and protein expression pattern was consistent. Based on our data and previous studies, we infer that the three elite factors Oct4, Nanog, and Sox2 formed their autoregulatory loops and maintained the pluripotent state of gHFSCs, as in ESCs. Self-renewal capacity of gHFSCs associated with the activity and expression of telomerase and AKP was higher than gADSCs. Higher expression of Oct4, Nanog, Sox2, AKP, and TERT in the gHFSCs indicated that the pluripotency and stemness of gHFSCs was stronger than gADSCs. We assume that HFSCs are similar to ESCs in the expression pattern of Oct4, Nanog, and Sox2 as these three transcription factors were all expressed in the gHFSCs both at RNA and protein levels, which should be further determined by

genome-wide analysis in the gHFSCs. In our previous study, we found an interesting phenomenon that the gHFSCs can form a spheroid structure when cultured in DMEM/F12 that is only supplemented with 10% FBS (S1). Furthermore, during the in vitro differentiation process, the cells also turned into a sphere shape as a transient or intermediate state, and as the differentiation is completed, the spheroid structure disappeared (S2). We suspected that this was related to the pluripotency of the gHFSCs.

Researchers obtain other adult stem cells such as adipose-derived, bone marrow-derived, or muscle-derived stem cells surgically. In comparison to these, HFSCs are easier to obtain with little trauma. In our study, the pluripotency and stemness of gHFSCs was stronger than gADSCs. Therefore, HFSCs could become a novel seed cell with more advantages for tissue engineering and clinical medicine. Also, gHFSCs can be a useful cell model to investigate genetic diversity in different types of hair follicles, further improving the quality and production of domestic animals.

Conclusions

A group of biomarkers, Oct4, Nanog, Sox2, AKP, and TERT, which are essential for establishment and maintenance of pluripotent state of the stem cells, were all expressed in the gHFSCs at the mRNA and protein level. When compared with gADSCs, their expression was significantly higher in the gHFSCs, suggesting the pluripotency and stemness of the gHFSCs were stronger than that of the gADSCs. Moreover, HFSCs have a good proliferative capacity with differentiation potential and are easy to obtain with minimal trauma; therefore, HFSCs are a reliable source of stem cells not only in biomedicine and dermatology but also in domestic animal production.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Amoh Y, Li L, Katsuoka K, Penman S, Hoffman RM (2005) Multipotent nestin-positive, keratin-negative hair-follicle bulge stem cells can form neurons. PNAS 102:5530–5534

Brett VJ, Rathjen J, Rathjen PD (2006) Transcriptional control of pluripotency: decisions in early development. Curr Opin Genet Dev 16:447–454

Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, Vrana J, Jones K, Grotewold L, Smith A (2007) Nanog safeguards pluripotency and mediates germline development. Nature 450: 1230–1234

Chen X, Xu H, Yuan P, Fang F (2008) Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133:1106–1117

Cotsarelis G, Sun T-T, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61:1329–1337

de Lange T (2009) How telomeres solve the end-protection problem. Science 326:948–952

Jaenisch R, Young R (2008) Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 132:567–582

Jimenez F, Izeta A, Poblet E (2010) Morphometric analysis of the human scalp hair follicle: practical implications for the hair transplant surgeon and hair regeneration studies. Dermatol Surg 37:58–64

Liu F, Uchugonova A, Kimura H, Zhang C, Zhao M, Zhang L, Koenig K, Duong J, Aki R, Saito N, Mii S, Amoh Y, Katsuoka K, Hoffman RM (2011) The bulge area is the major hair follicle source of nestin-expressing pluripotent stem cells which can repair the spinal cord compared to the dermal papilla. Cell Cycle 10(5):830–839

Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov A (2007) Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat Cell Biol 9:625–635

Niwa H, Masui S, Chambers I, Smith AG, Miyazaki J (2002) Phenotypic complementation establishes requirements for specific POU domain and generic transactivation function of Oct-3/4 in embryonic stem cells. Mol Cell Biol 22:1526–1536

Nowak JA, Fuchs E (2009) Isolation and culture of epithelial stem cells. Methods Mol Biol 482:215–232

O'Connor MD, Kardel MD, Iosfina I, Youssef D, Lu M, Li MM, Vercauteren S, Nagy A, Eaves CJ (2008) Alkaline phosphatase-positive colony formation is a sensitive, specific, and quantitative indicator of undifferentiated human embryonic stem cells. Stem Cells 26:1109–1116

Ohyama M, Terunuma A, Tock CL, Radonovich MF, Pise-Masison CA, Hopping SB, Brady JN, Udey MC, Vogel JC (2006) Characterization and isolation of stem cell-enriched human hair follicle bulge cells. J Clin Invest 116:249–260

Podlevsky JD, Chen JJL (2012) It all comes together at the ends: telomerase structure, function, and biogenesis. Mutat Res 730:3–11

Purba TS, Haslam IS, Poblet E, Jiménez F, Gandarillas A, Izeta A, Paus R (2014) Human epithelial hair follicle stem cells and their progeny: current state of knowledge, the widening gap in translational research and future challenges. Bioessays 36:513–525

Ren Y, Wu H, Wang X, Xue N, Liang H, Liu D (2014) Analysis of the stem cell characteristics of adult stem cells from Arbas white Cashmere goat. BBRC 448:121–128

Schefe JH, Lehmann KE, Buschmann IR, Unger T, Funke-Kaiser H (2006) Quantitative real-time RT-PCR data analysis: current concepts and the novel "gene expression's C T difference" formula. J Mol Med 84:901–910

Shay JW, Wright WE (2010) Telomeres and telomerase in normal and cancer stem cells. FEBS Lett 584:3819–3825

Štefková K, Procházková J, Pacherník J (2015) Alkaline phosphatase in stem cells. Stem Cells Int 2015:628368

Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM (2000) Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102:451–461

Trounson A, Thakar RG, Lomax G, Gibbons D (2011) Clinical trials of stem cell therapies. BMC Med 9:52

Yamanaka S, Takahashi K (2006) Induction of pluripotent stem cells from mouse fibroblast cultures. Tanpakushitsu Kakusan Koso 51:2346–2351

Yashiro M, Mii S, Aki R, Hamada Y, Arakawa N, Kawahara K, Hoffman RM, Amoh Y (2015) From hair to heart: nestin-expressing hair-follicle-associated pluripotent (HAP) stem cells differentiate to beating cardiac muscle cells. Cell Cycle 14(14):2362–2366

Young RA (2011) Control of the embryonic stem cell state. Cell 144:940–954

Yu HFD, Kumar SM, Li L, Nguyen TK, Acs G, Herlyn M, Xu X (2006) Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am J Pathol 168:1879–1888