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Generation of two Induced pluripotent stem cell lines from umbilical Cord–Derived mesenchymal stem cells

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ABSTRACT

Induced pluripotent stem cells (iPSCs) are generated by reprogramming terminally differentiated somatic cells using specific transcription factors, thereby enabling triploblastic differentiation. Common somatic cell sources include peripheral blood mononuclear cells (PBMCs), skin fibroblasts, and renal epithelial cells. However, the terminal differentiation status of these source cells introduces uncertainties in subsequent iPSC differentiation processes. In this study, umbilical cord–derived mesenchymal stem cells (UC-MSCs) were reprogrammed into iPSCs to explore an alternative cell origin. In vitro and in vivo assays confirmed that UC-MSC–derived iPSCs possess robust multidirectional differentiation potential, thereby providing solid experimental evidence supporting their application in further medical research.

1. Resource Table

Unique stem cell line identifier	SZBKi006-A SZBKi006-B
Alternative names of stem cell lines	N/A
Institution	Shenzhen Beike Biotechnology Co., Ltd
Contact information of distributor	Yanhong Cheng,R&D Center, Shenzhen Beike Biotechnology Co., Ltd,yanhong@beike.cc
Type of cell lines	iPSC
Origin	Human
Additional origin information	Ethnicity: Asian
Cell Source	umbilical cord mesenchymal stem cells (UC-MSC)
Clonality	Clonal
Method of reprogramming	Episomal plasmids reprogramming
Genetic modification	N/A
Type of modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/Constitutive system	N/A
Date archived/stock date	12/2023
Cell line repository/bank	https://hpscreg.eu
Ethical approval	Ethics Committee of Shenzhen Beike Biotechnology Co., Ltd.

2. Resource utility

The SZBKi006-A and SZBKi006-B cell lines were derived from the same UC-MSC population. These iPSC cell lines can be applied to novel drug development, drug toxicology screening, and pharmalogical research (Nicholson et al., 2022; Shi et al., 2017). Moreover, these iPSCs may also contribute to clinical cell therapy applications, including autologous cell transplantation (Song et al., 2020) and gene-modified

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cell therapy(Maxwell and Millman, 2021). Currently, most iPSC lines are derived from PBMCs, skin fibroblasts, or urine; however, iPSCs generated from different somatic cell sources exhibit different differentiation tendencies. Unlike these somatic cell-derived iPSCs, SZBKi006-A and SZBKi006-B were reprogrammed from UC–MSCs, which intrinsically possess multilineage differentiation potentials; therefore, these UC-MSC–derived iPSC lines may be particularly valuable for subsequent scientific studies (Pichard et al., 2021; Miere et al., 2016).

3. Resource details

The MSCs used in this study were isolated from the umbilical cord of a newborn infant. Following overexpression of reprogramming factors in expanded UC-MSCs, colonies morphologically resembling pluripotent stem cells were isolated after 30 days. Fig. 1 exhibited the morphology of the reprogrammed iPSC lines after colony purification. Two reprogrammed colonies were purified and designated SZBKi006-A and SZBKi006-B, respectively. The expression levels of core pluripotency–related genes in SZBKi006-A and SZBKi006-B were tested assessed RT-PCR and showed a significant increase compared with the corresponding UC-MSCs (Fig. 1B). These results were further validated using flow cytometry and immunofluorescence assays, which demonstrated high expression of the core pluripotency–related proteins SOX2, OCT4, and NANOG in reprogrammed iPSCs (Fig. 1D, E). Prio to triploblastic differentiation assays, the karyotypes of both iPSC lines were examined, and the results confirmed that SZBKi006-A and SZBKi006-B exhibited a normal karyotype (Fig. 1C). Short tandem repeat (STR) (the above-mentioned materials have been submitted to the journal archive) analysis confirmed that both SZBKi006-A and SZBKi006-B shared genetic profiles with the corresponding UC-MSCs.

The differentiation potential of iPSCs was assayed using a trilineage differentiation kit, and flow cytometry results showed clear expression of specific markers in each germ layer, demonstrating that the iPSCs could differentiate into all three germ layers (Fig. 1F). In addition, a teratoma assay was performed to verify the triploblastic differentiation capacity of SZBKi006-A and SZBKi006-B *in vivo*. Photographs in Fig. 1G show representative tissues from each germ layer, further confirming the differentiation capacity of the reprogrammed iPSC lines.

Furthermore, additional lineage-specific markers were detected in each germ layer using RT-PCR. Fig. 1H provides strong evidence that the generated iPSCs are capable of trilineage differentiation. Genomic polymerase chain reaction analysis further confirmed that no residual episomal plasmids were detected in either iPSC line (shown in Fig. 1I).

4. Materials and methods

4.1. Generation of iPSCs from UC-MSC

Isolation and culture of UC-MSCs were performed as previously described by Yang et al(Yang et al., 2022).Before reprogramming, the cells were analyzed by flow cytometry and were confirmed to express characteristic UC-MSC markers. Collected UC-MSCs were cultured in Nutristem MSC XF Basal Medium (Biological Industries, Cat#05–200-1A) supplemented with Nutristem MSC XF Supplement Mix (Biological Industries, Cat# 05–201-1U) and 5% Stemulate Xeno- and heparin-free pooled platelet lysate (Sexton Biotechnologies, Cat# PL-SP-100).UC-MSCs were electroporated with four episomal plasmids (Miaoling #P0500, #P0501, #P0502, and #P0503, China) according to the manufacturer's instructions for the Human CD34 + Cell NucleofectorTM Kit (Lonza Cat# VPA-1003, Switzerland).

At passage 2 (P2), electroporation was performed on 2×10^6 cells using a NEPA21 electroporator at 200 V with 50 pulses for 5 ms each. Transfected cells were cultured on Matrigel-coated plates (Corning Incorporated Cat# 354277) in Nutristem MSC XF Basal Medium supplemented with Nutristem MSC XF Supplement Mix and 5% Stemulate Xeno- and heparin-free pooled platelet lysate. On day 6, the medium was replaced with ReproTeSR™ Reprogramming Medium (STEMCELL Technologies, Cat# 05920). After an additional 5 days, the medium was changed to mTeSR1 Medium (STEMCELL Technologies, Cat# 85850). The medium was changed daily until the iPSC colonies were manually picked. When passaging was required, clones were split at a ratio of 1:3–1:5 using Accutase (STEMCELL Technologies, Cat# 07920). Cells were passaged every 3–4 days. On the first day after seeding, a ROCK inhibitor was added at a final concentration 10 μm/mL, followed by culture at 37 °C in an incubator with 5% carbon dioxide, with daily medium changes.

4.2. Total RNA extraction and Real-time PCR

Total RNA from iPSCs was extracted using the Rneasy Mini kit (QIAGEN, Cat# 74104, Germany) and the concentration was determined using a Nanodrop spectrophotometer (Thermo Scientific, USA). Total RNA was reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit (TAKARA, Japan). RT-PCR assays were performed according to the manufacturer's instructions provided with the RT-PCR kit (TOYOBO, Japan). DNA amplification was carried out using specific sense and antisense primers, and the primer sequences are listed in Table 2.

4.3. Flow cytometry assay

Before analysis, iPSCs were harvested, fixed, permeabilized, and incubated with antibodies at 4 °C for 40 min according to the protocol provided with the Transcription Factor Buffer Set (BD Pharmingen, Cat# 562574). Samples were subsequently analyzed using a BD FACS Canto II flow cytometer. The antibodies used in this study are listed in Table 1.

4.4. Immunofluorescence assay

iPSCs were fixed with 4% paraformaldehyde for 15 min at 4 °C and then washed three times with DPBS. After permeabilization and blocking for 30 min at room temperature, the cells were incubated with primary antibodies overnight at 4 °C. The following day, the cells were washed three times with DPBS and subsequently incubated with secondary antibodies for 2 h at room temperature. Cell nuclei were then stained with DAPI, and images were acquired using an Olympus IX83 fluorescence at room temperature.

4.5. Karyotype analysis and STR analysis

Karyotype and STR analyses of all iPSC lines were performed by Shenzhen Kenuo Medical Laboratory at passage 15 (P15). Chromosome G-banding analysis was conducted using a Zeiss Ikaros karyotype analysis system. PCR-STR genotypes were analyzed using an ABI 3730XL DNA analyzer.

4.6. Trilineage differentiation in vitro

Trilineage differentiation of iPSC lines was performed according to the manufacturer's instructions for the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies, Cat# 05230) at passage 12 (P12). Flow cytometry and immunofluorescence assays were used to assess the differentiation into each germ layer. Antibodies specific to markers of each germ layer were selected for detection following incubation with differentiated cells. The antibodies used for trilineage differentiation analysis are listed in Table 2. Cell nuclei were stained with DAPI, and immunofluorescence images were captured using an Olympus IX83 fluorescence microscope. Flow cytometry data were analyzed using the FlowJo software.

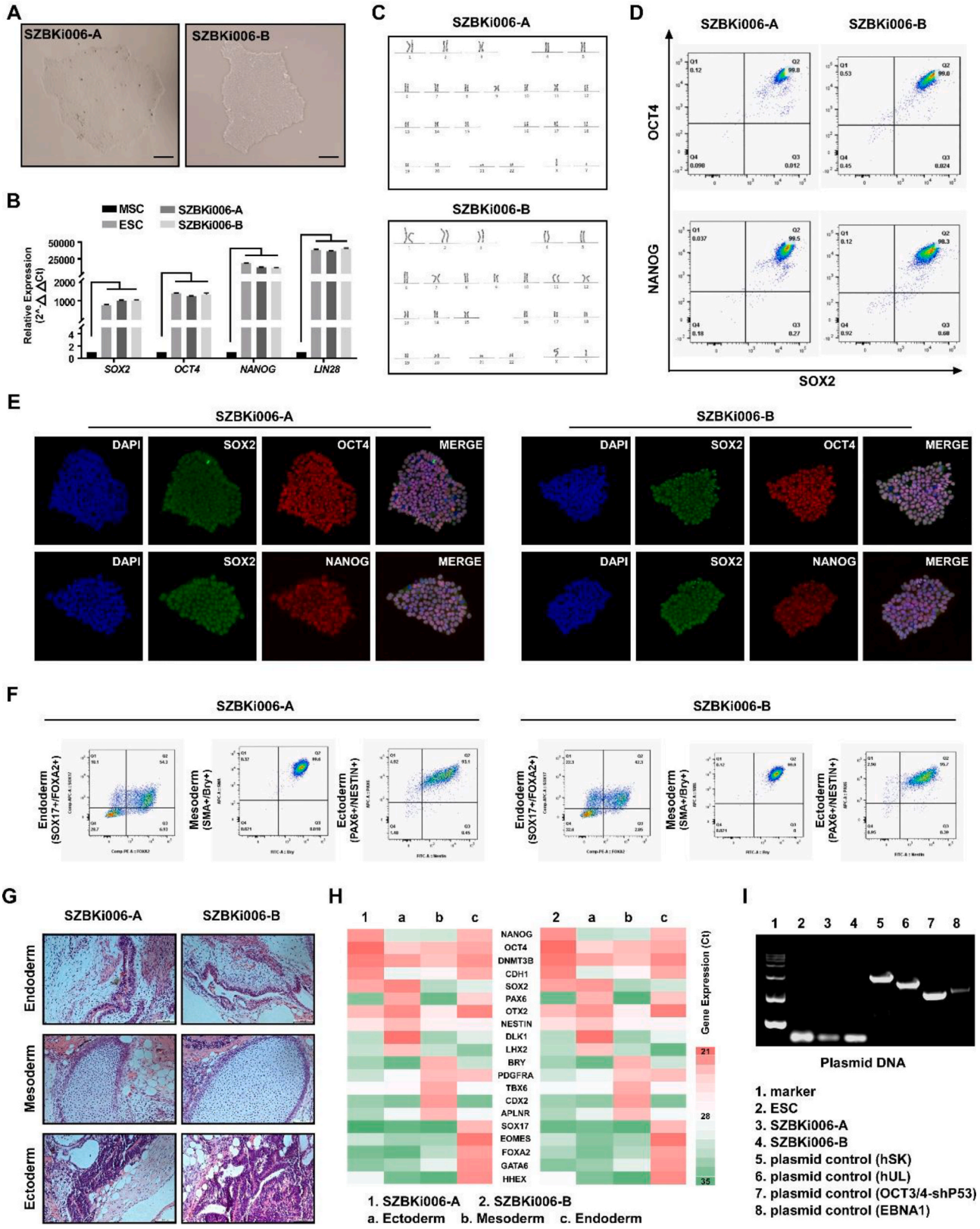

Fig. 1. A. Cell morphology of two reprogramming induced pluripotent stem cells. B. RT-PCR data showing pluripotent markers expression in iPSCs, UC-MSC was act as control (n = 3). C. G-banding assay showing all the iPSCs had a normal karyotype. D. Flow cytometry result showing two iPSCs exhibiting high expression with pluripotent markers. E. Immunofluorescence assay illustrating iPSCs had a high expression of the pluripotent markers. F. Flow cytometry data indicating all the iPSCs had the trilineage differentiation ability. G. Teratoma assay revealed that the iPSCs could differentiate into the cells in all three germ layers in vivo (n = 3). H. Molecular analysis of cultures differentiated with different germ layer differentiation medium, key germ layer markers were tested by RT-PCR and the Ct values were exhibited by hot map. I. The non-integration of episomal plasmids were determined by genomic PCR.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	RT-PCR	Expression of endogenous core pluripotency related gene markers: SOX2, OCT4, NANOG and Lin28	Fig. 1B
	Immunofluorescence Assay	Positive for core pluripotency related protein markers of human stem cells: SOX2, OCT4, NANOG	Fig. 1E
	Flow cytometry	SOX2+/ OCT4+>90%; SOX2+/ NANOG+>90%	Fig. 1D
Genotype	Karyotype (G-banding)	46XY (male); 46XX (female)	Fig. 1C
Identity	STR analysis	N/A	Available with authors submitted to the journal archive
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma(P15)	Mycoplasma testing by RT-PCR	Available with authors
Differentiation potential	Triple germ layer differentiation	Positive for Ectoderm: PAX6+/Nestin+; Endoderm: SOX17+/ FOXA2+; Mesoderm: Brachyury+/ α-SMA+	Fig. 1F
	Teratoma assay	Histological staining for representative cell types for ectoderm, mesoderm, and endoderm.	Fig. 1G
List of recommended germ layer markers	Expression of these markers must be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: PAX6+/Nestin+ Endoderm: SOX17+/FOXA2+ Mesoderm: Brachyury+/ α-SMA+	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Markers of core protein	anti-OCT4	1:200	Abcam, Cat#ab181557	RRID: AB_2687916
	Anti-SOX2	1:100	Abcam, Cat#ab79351	RRID: AB _10710406
	Anti-NANOG	1:200	Cell Signaling Technology, Cat#4903	RRID: AB _10559205
Differentiation Markers	Anti-PAX6	1:200	Cell Signaling Technology, Cat#60433	RRID: AB_2797599
	Anti-Nestin	1:200	Abcam, Cat#ab18102	RRID: AB_444246
	Anti-Brachyury	1:500	Abcam, Cat#ab209665	RRID: AB_2750925
	Anti-α-SMA	1:500	Abcam, Cat#ab7817	RRID: AB_262054
	Anti-SOX17	1:100	R&D Systems, Cat#AF1924	RRID: AB_355060
	Anti-FOXA2	1:100	Abcam, Cat#ab60721	RRID: AB_941632
Secondary antibodies	Goat Anti-Mouse IgG (H + L) Antibody, Alexa Fluor 488	1:1000	Thermo Fisher Scientific, Cat#A-11029	RRID: AB_2534088
	Goat Anti-Mouse IgG (H + L) Antibody, Alexa Fluor 568	1:1000	Thermo Fisher Scientific, Cat#A-11004	RRID: AB_2534072
	Goat anti-Rabbit IgG (H + L) Antibody, Alexa Fluor 488	1:1000	Thermo Fisher Scientific, Cat#A-11034	RRID: AB_2576217
	Donkey anti-Rabbit IgG (H + L) Antibody, Alexa Fluor 568	1:1000	Thermo Fisher Scientific, Cat#A-10042	RRID: AB_2534017
	Donkey anti-Goat IgG (H + L) Antibody, Alexa Fluor 488	1:1000	Thermo Fisher Scientific, Cat#A-11055	RRID: AB_2534102
	Goat anti-Rabbit IgG (H + L) Antibody, Alexa Fluor 647	1:1000	Thermo Fisher Scientific, Cat#A-21245	RRID: AB_2535813
PCR Primers				
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal Plasmids	Four transfection plasmids	4610 bp	GCAACGTGCTGGTTATTGTG/ CATAGCGTAAAAGGAGCAACA	
Markers of core genes	*OCT4*	106 bp	CCTGAAGCAGAAGAGGATCACC/ AAAGCGGCAGATGGTCGTTTGG	
	SOX2	135 bp	GCTACAGCATGATGCAGGACCA/ TCTGCGAGCTGGTCATGGAGTT	
	NANOG	115 bp	CTCCAACATCCTGAACCTCAGC/ CGTCACACCATTGCTATTCTTCG	
	LIN28	125 bp	CCAGTGGATGTCTTTGTGCACC/ GTGACACGGATGGATTCCAGAC	
House-Keeping Genes	*GAPDH*	131 bp	GTCTCCTCTGACTTCAACAGCG/ ACCACCCTGTTGCTGTAGCCAA	

4.7. Teratoma formation assay

iPSCs were expanded and resuspended with 100 μL of highly concentrated Matrigel (Corning, Cat# 354262) and then injected into the unilateral axillary subcutaneous region of NOD-SCID mice at a dose of 2×10^6 cells per mouse at passage 5 (P5). All teratomas formed within 8 weeks, after which the mice were euthanized and the teratomas were harvested for paraffin embedding and hematoxylin and eosin (H&E) staining. The *in vivo* differentiation potential of iPSCs was evaluated

based on histological analyses.

4.8. Genomic DNA analysis

Genomic DNA from iPSC lines was isolated using a genomic DNA extraction kit (TIANGEN Biotech, China) at passage 12 (P12). The sequences of the polymerase chain reaction primers are listed in Table 2.

5. Discussion

Although iPSC-related research has gradually matured and numerous iPSC technologies have been developed, substantial challenges and uncertainties remain for their clinical application, including tumorigenicity, genetic mutations, immune rejection, and differentiation instability(Madrid et al., 2021). Differentiation bias is another important consideration. iPSCs tend to preferentially differentiate toward their cells of origin during differentiation, depending on the somatic cell from which they are derived. In this study, UC-MSCs were selected as the starting cell source for iPSC reprogramming. MSCs, unlike most other somatic cell types, are stem cells with intrinsic multilineage differentiation potential. Therefore, this study provides an alternative perspective on iPSC cell sources and lays the foundation for further in-depth investigations into the functional properties and applications of MSC-derived iPSCs.

CRediT authorship contribution statement

Yanhong Cheng: Methodology, Investigation. **Jingyu Xu:** Validation, Data curation. **Junjie Wen:** Validation, Investigation. **Dan Xiao:** Validation, Methodology. **Liang Wang:** Data curation. **Ran Zheng:** Writing – review & editing. **Cheguo Cai:** Writing – review & editing, Supervision. **Yunfeng Gao:** Writing – original draft, Supervision, Project administration, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2026.103954.

Data availability

Data will be made available on request.

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