Original Research

Stemness gene expression profile analysis in human umbilical cord mesenchymal stem cells

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Abstract

Umbilical cord mesenchymal stem cells (UC-MSCs) have several advantages for clinical therapy: the material is easily obtainable, the donation procedure is painless and there is low risk of viral contamination. UC-MSCs play important roles in tissue regeneration, tissue damage repair, autoimmune disease and graft-versus-host disease. In this study, we investigated the normal mRNA expression profile of UC-MSCs, and analyzed the candidate proteins responsible for the signaling pathway that may affect the differentiation characteristics of UC-MSCs. UC-MSCs were isolated by mincing UC samples into fragments and placing them in growth medium in a six-well plate. The immunophenotype characteristics and multilineage differentiation potential of the UC-MSCs were measured by flow cytometry and immunohistochemical assays. In addition, the pathway-focused gene expression profile of UC-MSCs was compared with those of normal or tumorous cells by realtime quantitative polymerase chain reaction. We successfully isolated and cultured UC-MSCs and analyzed the appropriate surface markers and their capacity for osteogenic, adipogenic and neural differentiation. In total, 168 genes focusing on signal pathways were examined. We found that the expression levels of some genes were much higher or lower than those of control cells, either normal or tumorous. UC-MSCs exhibit a unique mRNA expression profile of pathway-focused genes, especially some stemness genes, which warrants further investigation.

Keywords: mesenchymal stem cells, umbilical cord, realtime PCR, pathway-focused genes, therapeutic perspective

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Introduction

Mesenchymal stem cells (MSCs) originate from the mesenchyme and are a type of adult stem cell. They have multidifferentiation potential; that is, they are capable of differentiation into various tissues of mesenchymal and endodermal origin. MSCs exhibit low immunogenicity and immune regulation capability, and are not damaged or killed by cytotoxic T lymphocytes or natural killer (NK) cells.[1,2] Since they have strong tissue repair capability, they provide trophic support and modulate the innate immune response. Thus, MSCs are attractive for clinical therapy and play important roles in tissue regeneration, tissue damage repair, autoimmune disease and graft-versus-host disease.

MSCs are mainly isolated from the placenta, adipose tissue, lung, bone marrow (BM), blood, umbilical cord (Wharton's jelly) and teeth (the perivascular niche within dental pulp and the periodontal ligament).[3,4] BM is a major source of MSCs, and has been widely used in a variety of experimental or clinical studies. However, one of the major difficulties with BM is its collection, which involves a very invasive procedure to the donors. There is also a marked decline in cell number, proliferation capacity and differentiation potential of BM with age.[5] Therefore, an alternative source to BM for MSC isolation would be invaluable for future cell therapy.

Previous studies have shown that placenta and Wharton's jelly from umbilical cord (UC) are the other two main sources of MSCs in the body.[6] Of those two tissues, the UC is usually more easily obtained than the placenta, and can be collected after birth with the mother's consent. Thus, since UCs are easily obtainable, involve no pain to donors and have a lower risk of viral contamination, they are promising sources for autologous cell therapy. Some reports have suggested that UC-MSCs represent a promising alternative cell source for MSC-based therapies, as they are incapable of expressing major histocompatibility complex class II (human leukocyte antigen [HLA)-DR]) antigens,[7] and thus there is no need for immune suppression.

Some recent studies have investigated the use of cell-based therapy with UC-MSCs for stroke,[8] retinal degeneration[9] and hemiparkinsonism.[10] UC-MSCs have been widely investigated for their multipotent differentiation *in vitro* into osteogenic, adipogenic, neural, hepatocyte-like and endothelial-cells.[11–14] However, the molecular and cellular mechanisms underpinning these associated changes remain unclear. In addition, little is known about the basic biological characteristics of UC-MSCs, especially their stemness gene expression profile.

To provide basic information about the normal gene expression profile of UC-MSCs, and to analyze candidate proteins responsible for the signaling pathway that may affect the differentiation characteristics of UC-MSCs, we used a realtime (RT)-polymerase chain reaction (PCR) to compare the gene transcript profile between UC-MSCs, an embryonic cell line and two tumor cells. Precise evaluation of the different pathway-focused gene expression levels between normal cell and tumor cells should greatly help in elucidating the roles of UC-MSC proteins in tissue repair and differentiation, and provide basic gene expression data that will provide insight into the use of UC-MSCs as a promising source for cell therapy.

Materials and methods

Isolation and culture of human UC-MSCs

UC-MSC culture

Human UCs were collected from women (negative for human immunodeficiency virus, hepatitis B and C viruses and syphilis) who had given birth at Yan'an Hospital of Kunming Medical University. (During this study, informed consent in writing was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1989 Declaration of Helsinki; this study was conducted after approval from the ethics committee of Yan'an Hospital of Kunming Medical University.) The samples were collected aseptically and washed thoroughly with phosphate-buffered saline (PBS) with antibiotics (100 IU/mL ampicillin, 100 μg/mL streptomycin) to remove contamination. Two arteries and one vein in the UCs were carefully dissected from Wharton's jelly. The jelly was then cut into fragments of approximately 1 mm^3, and placed in a six-well plate with α-modified Eagle's medium (α-MEM) (Life Technologies, Carlsbad, CA, USA) supplemented with 20% (v/v) fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT, USA), 100 U/mL penicillin and 100 mg/mL streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C. After three days of culture, the medium was replaced, and non-adherent cells were removed. The medium was then changed twice weekly thereafter. Once 60–80% confluence was achieved, adherent cells were digested with 0.25% trypsin for proliferation (generation 4). UC-MSCs at the third or fourth passage were used for all experiments.

Control cell line culture

A human embryonic fibroblast lung diploid cell strain, KMB-17, was obtained as a normal cell control (Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Kunming, China). Cells at passage 24 were used in this study. A human hepatoma cell line, BEL-7404, and a human lung adenocarcinama cell line, SPC-A-1, were purchased from the cell bank of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China and used as tumor cell controls. These cells were maintained in RPMI 1640 medium (Hyclone Laboratories) supplemented with 10% (v/v) FBS and antibiotics (100 IU/mL penicillin, 100 mg/mL streptomycin).

Analysis of cell surface markers

Cultures were harvested by 0.05% trypsin/ethylenediaminetetraacetic acid (Invitrogen Corp., Carlsbad, CA, USA) and washed twice with PBS. Cells were then adjusted to a density of 1×10^7/mL in PBS supplemented with 1% bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA). Cell suspensions (100 μL) were incubated with 20 μL fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mouse anti-human monoclonal antibodies for 30 min at room temperature in the dark. The antibodies were HLA-DR-FITC, CD45RA-FITC, CD34-FITC, CD45RO-PE, CD45RB-PE, CD123-FITC, CD166-PE, CD49b-FITC and CD73-PE (Becton Dickinson, Franklin Lakes, NJ, USA). After washing with PBS, cells were processed through a three-color flow cytometer (FACScalibur; Becton Dickinson), and analyzed by CellQuest software (Becton Dickinson). Non-specific background staining was evaluated by parallel staining with isotype-matched IgG1-FITC and IgG1-PE.

Osteogenic, adipogenic and neural differentiation

Osteogenic differentiation and adipogenic differentiation

Briefly, 2 mL of UC-MSC suspension (5×10^4 cells) were seeded into a six-well plate and incubated at 37°C in α-MEM medium for 24 h. The supernatant was discarded and the growth medium changed to a medium appropriate for assaying. The osteogenic differentiation medium consisted of Dulbecco's modified Eagle's medium with low glucose (DMEM-LG) supplemented with 10% FBS, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, 100 nmol/L dexamethasone, 0.2 mmol/L L-ascorbate and 10 mmol/L beta-glycerophosphate (Sigma-Aldrich). The adipogenic differentiation medium consisted of DMEM supplemented with 10% FBS, 10 mmol/L glycerophosphate, 100 nmol/L dexamethasone and 0.2 mmol/L ascorbic acid 2-phosphate. For both, the medium was changed every three days. Osteogenic and adipogenic differentiation were checked by von Kossa staining every two weeks.

Neural differentiation

UC-MSCs were added to six-well plates at a density of 1×10^4 cells/cm^2 and incubated at 37°C in α-MEM medium for 24 h. The supernatant was discarded and replaced with induction medium, consisting of DMEM supplemented with 2% (v/v) FBS, 10 ng/mL epidermal

growth factor, 1 g/L glucose, 10 ng/mL platelet-derived growth factor, 1×10^{-7} mol/L hexadecadrol, 0.5 μmol/L linoleic acid and 50 μg/mL gentamicin (all from R&D Systems, Minneapolis, MN, USA). The medium was changed every three days for two weeks. Neural differentiation was assessed by antibodies against human neurofilament M, synaptophysin, tubulin and galactocerebroside (all from Chemicon, Temecula, CA, USA) and PE-conjugated goat Ab anti-mouse immunoglobulins.

Realtime quantitative PCR

UC-MSCs were harvested when they reached 90% confluence. Total RNA was extracted (TRI reagent; Invitrogen Corp.) and then subjected to reverse transcription with RevertAid^{TM} H Minus First Strand cDNA Synthesis Kit (Fermentas, Glen Burnie, MD, USA). Then, using two pathway-focused gene expression profiling kits (RT2 Profiler PCR Array System; SABiosciences Corporation, Frederick, MD, USA), cDNAs were added to 96-well plates. Realtime quantitative PCR was carried out on an automated realtime-PCR system (ABI 7300; Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The worksheet of the gene expression profiling kit included analysis of the positive PCR, reverse transcription and genomic DNA controls. The formula used to calculate the relative gene expression level ($2^{-\Delta Ct}$) in the 'Results' worksheet is: $\Delta Ct = Ct$ (GOI) – avg (Ct [HKG]), where GOI is each gene of interest and HKG are the housekeeping genes chosen for the 'Sample1-ControlGene' worksheet. Calculations of averages were performed and are shown in the calculations worksheet.

Statistical analysis

Statistical analysis was performed using independent samples *t*-test (SPSS 13.0, see http://www-01.ibm.com/software/analytics/spss). Results are expressed as mean $\pm$ SD. $P \leq 0.05$ was considered significant.

Results

Cellular isolation, culturing and morphology of UC-MSCs and control cells

UC-MSCs isolated from human Wharton's jelly and used in the present work displayed a consistent spindle-shaped, elongated morphology similar to fibroblast cells, with abundant cytoplasm and large nucleoli (Figure 1). KMB17 cells had a spindle-like shape and were arranged in closely packed sheets and islands. Microscopic examination of the tumor cells, BEL-7404 and SPC-A-1, showed that they

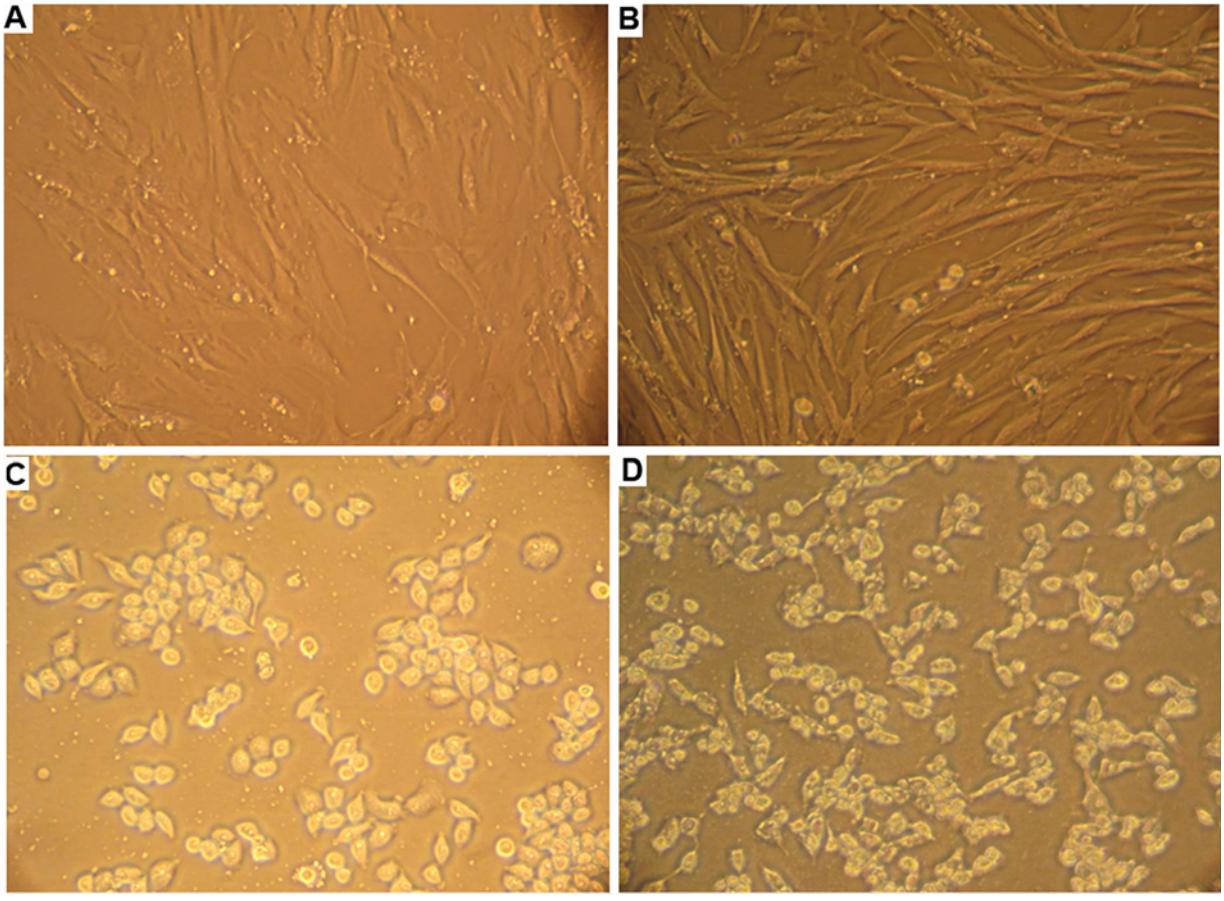

Figure 1 The morphology of umbilical cord mesenchymal stem cells (UC-MSCs) and other control cells (phase contrast microscope; Olympus, Tokyo, Japan; ×400). (a) UC-MSCs displayed a consistent spindle shape, with abundant cytoplasm and large nucleoli. (b) KMB17 cells were arranged in closely packed sheets and had a spindle-like shape. (c) SPC-A-1 cells were composed of large polygonal cells with large central eosinophilic nucleoli and occasional mitotic figures. (d) Similarly, BEL-7404 cells were present as polygonal cells with vesicular nuclei

Table 1 The immunophenotype of UC-MSCs was different from that of the other cell types

Gene	UC-MSC	KMB17	BEL-7404	SPC-A-1
CD123	4.94	22.71	3.21	91.72
CD166	99.41	97.45	98.38	87.30
CD49b	95.19	99.65	79.89	94.10
CD73	97.42	93.46	91.49	15.63
CD29	92.80	99.95	99.76	100
HLA-DR	3.88	15.00	0.97	33.06
CD45RA	0.37	2.26	2.57	6.77
CD45RB	0.32	1.10	0.29	0.37
CD34	0.13	1.48	1.04	0.35
CD45RO	0.41	1.45	2.74	0.24

Data are given as percentage of cells
UC-MSC, umbilical cord mesenchymal stem cell; KMB17, human embryonic fibroblast lung diploid cell strain; BEL-7404, human hepatoma cell line; SPC-A-1, human lung adenocarcinama cell line

were composed of large polygonal cells; most had vesicular nuclei with large central eosinophilic nucleoli, and occasional mitotic figures were present. These features were maintained in all cell types to the end of cell culturing.

Immunophenotypic potential of UC-MSCs and control cells

As revealed by flow cytometry, UC-MSCs expressed hemic markers such as CD73, CD29, CD166 and CD49b. However, the expression of CD123, HLA-DR, CD45RA, CD45RB and the leukocytic marker CD34 was significantly lower in UC-MSCs, whereas the expression of HLA-DR and CD123 was high in SPC-A-1 tumor cells (Table 1 and Figure 2).

Differentiation capabilities of UC-MSCs

Differentiation of UC-MSCs was evaluated using P4 cells derived from UCs. Cells maintained in growth medium were used as negative controls. After adipogenic and osteogenic induction, UC-MSCs could differentiate into osteoblasts, and using von Kossa staining, formation of a mineralized matrix of UC-MSCs samples could be detected (Figure 3a). Oil Red O staining was positive for neutral lipid vacuoles in UC-MSCs (Figure 3c), indicating that these cells could differentiate into adipocytes. Neither osteoblasts nor adipocytes were found in control cells maintained in regular growth medium. Using immunofluoresence microscopy, UC-MSCs displayed a neuron-like morphology, with cells positive for markers of galactocerebriside, M-nerve plexus, synaptophysin and microtubulin, indicating that UC-MSCs could differentiate into neural cells. No expression of the specific marker for neural differentiation was seen in cells growing in regular medium (Figure 4).

Pathway-focused gene expression profile analysis was different between UC-MSCs and other cell lines

To determine the pathway-focused gene expression profile, RT-PCR was performed on UC-MSCs (P4), KMB17 (P24), BEL-7404 and SPC-A-1 in 96-well plates. We examined 168 genes, focusing on the cellular signaling pathways and differential gene expression levels between UC-MSCs, KMB17, BEL-7404 and SPC-A-1 (Supplementary Table 1); please see http://ebm.rsmjournals.com/lookup/suppl/doi:10.1258/ebm.2012.011429/-/DC1. Differences between UC-MSCs and the other three cell types of 10-fold or greater were listed and selected for further analysis (Tables 2 and 3).

Higher gene (stemness gene) expression profile in UC-MSCs

The pathway-focused gene overexpression profile was found to be different between UC-MSCs and the other three cell types. Fifteen genes had a 10-fold higher

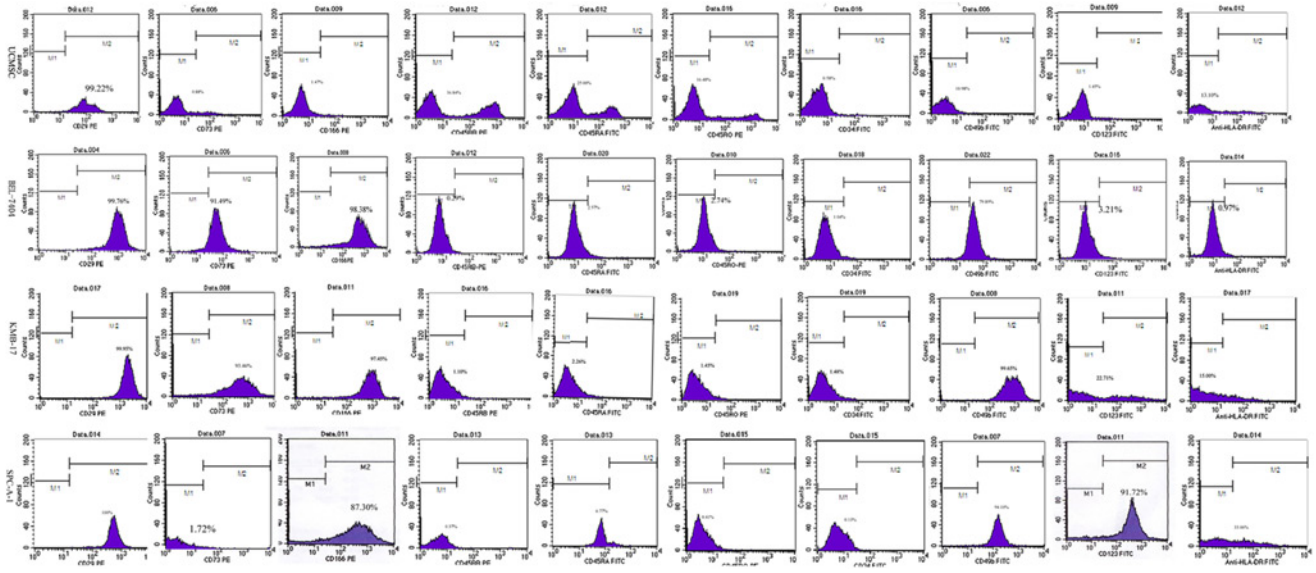

Figure 2 Detection of mesenchymal stem cell (MSC)-specific marker expression by flow cytometric analysis. M1 represents an antibody isotype control for background fluorescence and M2 shows the signal from cell surface marker antibodies. Umbilical cord (UC)-MSCs and the control cells KMB17, BEL-7404, BEL-7404 and SPC-A-1 were stained with HLA-DR-FITC, CD45RA-FITC, CD34-FITC, CD45RO-PE, CD45RB-PE, CD123-FITC, CD166-PE, CD49b-FITC and CD73-PE. (A color version of this figure is available in the online journal)

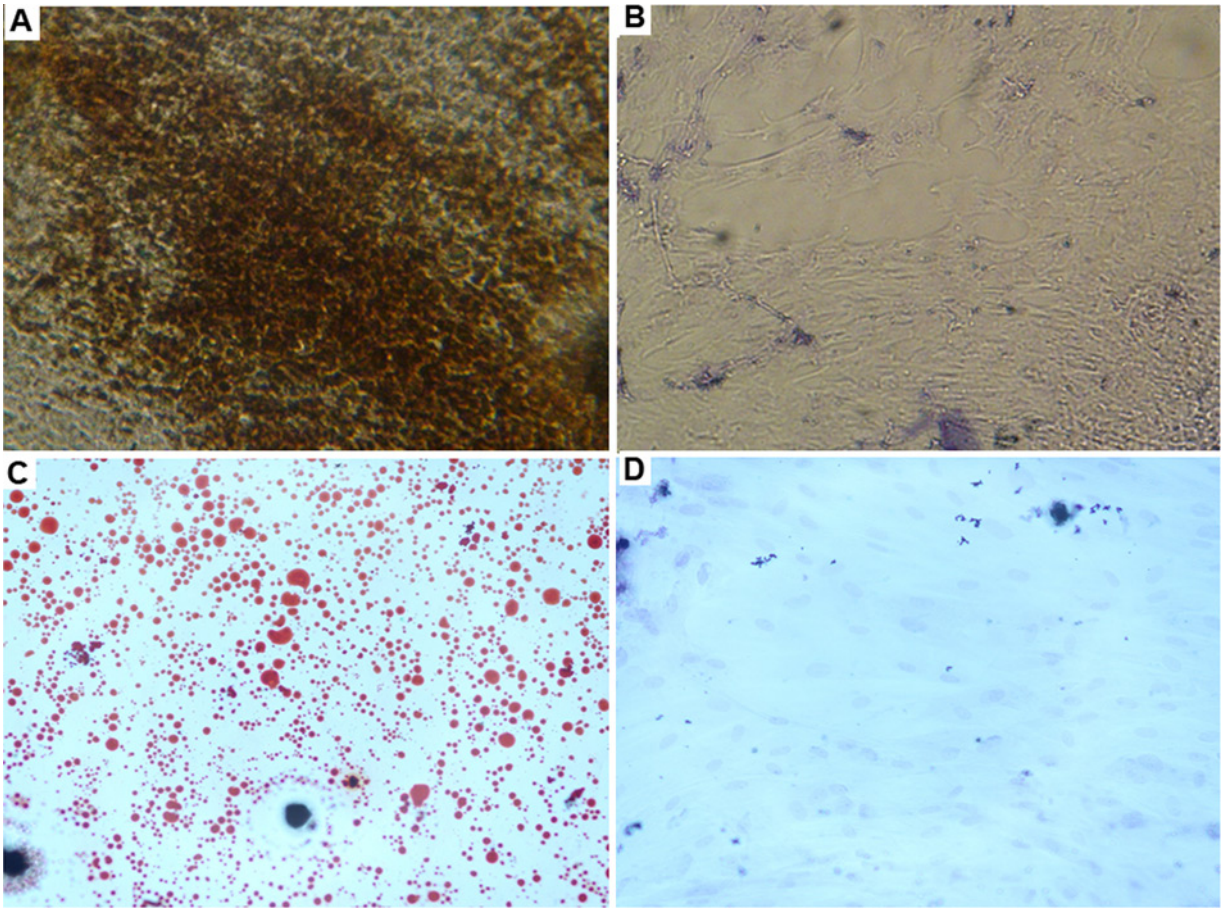

Figure 3 Differentiation potential of umbilical cord mesenchymal stem cells (UC-MSCs) (phase contrast microcope; Olympus; ×100). (a) Cultured in osteogenic medium, UC-MSCs could differentiate into osteoblasts as seen by von Kossa staining. (b) Parallel controls in the growth medium. (c) Cultured in adipogenic medium, UC-MSCs could differentiate into adipocytes as seen by Oil Red O staining. (d) The controls could not change into adipocytes in normal growth medium

expression level in UC-MSCs than in KMB-17 cells. These genes could be divided into three categories: (1) stem cell-specific markers (*CDC2, FGF1, NEUROG2, FGF2, JAG1, ALDH2, ADAR*); (2) stem cell differentiation markers (*KRT15, ALPI, COL1A1, CD44, NCAM1*); and (3) signaling proteins important for stem cell maintenance (*DTX2, DVL1, NUMB*) (Table 2). Of these, *CD44*, *COL1A1*, *DTX2* and *NEUROG2* had the highest mRNA expression levels, which were at least a 1000-fold higher in UC-MSCs than in KMB17 cells. Similarly, 12 genes had expression levels at least 10-fold higher in UC-MSCs than in SPC-A-1 cells, including six stem cell-specific markers (*CCND1, CDC2, HSPA9, BMP2, CTNNA1* and *SOX2*), five stem cell-specific markers (*FOXA2, ISL1, COL1A1, MME* and *ALP1*) and one signaling pathway gene (*DTX2*). In addition, 13 genes had expression levels that were at least 10-fold higher in UC-MSCs compared with BEL-7404 cells, and included six stem cell-specific markers (*APC, CCND2, HSPA9, BMP2, JAG1, CD4* and *MYST1*) and four signaling pathway genes (*FOXA2, CD8A, COL1A1* and *MME*). The genes common to both SPC-A-1 cells and BEL-7404 tumor cell lines, which had higher expression in UC-MSCs, were *BMP2*, *COL1A1*, *DTX2*, *FOXA2*, *HSPA9* and *MME*. Notably, the genes common to all three cell types that had a higher expression in UC-MSCs were *COL1A1* and *DTX2* (Table 2).

Lower gene expression profile in UC-MSCs

In contrast, some genes had significantly lower expression levels in UC-MSCs compared with the other three cell types. These genes were mainly signal pathway ligands and cytokines, and again could be classified into three groups: (1) signal pathway ligands and cytokines (*IFNA, IFNG, IFNK, IL2, IL3, IL13, IL16, IL17, TGFB1, TNFSF11, TNFSF13 DLL3*, *HDAC2*, *PDX2* and *ISL1*); (2) genes associated with cell growth and differentiation (*APC*, *CCNE1*, *COL2A1*, *ASCL2*, *CDC42*, *ISL1*, *SOX1*, *ACTC1*, *GDF2*, *GDF3, BMP1, BMP8B, BMP5, FIGF, CSF1, CSF2, GDF11* and *GDF3*); and (3) tumor-related or suppressor genes (*APC*, *RB1*, *CDH1*). In total, genes which were expressed at levels 10-fold lower in UC-MSCs than in KMB17 cells including 27 signal pathway ligands and cytokines. When compared with SPC-A-1 cells, 22 signal pathway ligands and cytokine genes, were at least 10-fold lower in UC-MSCs. Similarly, 20 signal pathway ligands and cytokine genes had a lower expression in UC-MSCs than in BEL-7404 cells (Table 3). The genes common to both tumor cell lines that had a lower expression in UC-MSCs were *ACAN*, *CCNE1*, *CDH1*, *COL2A1*, *DLL3*, *MYST2*, *SOX1*, *FIGF*, *GDF11*, *GDF2*, *GDF3*, *RB1*, *IFNA1*, *IL17B*, *T*, *WNT1*, *GJB1*, *IL17C*, *IL1F7*, *IL2*, *IL5* and *TNFSF11*. There were 11 genes in common for all three cell types that had lower expression levels in UC-MSCs; namely, CCNE1, *CDH1*, *COL2A1*,

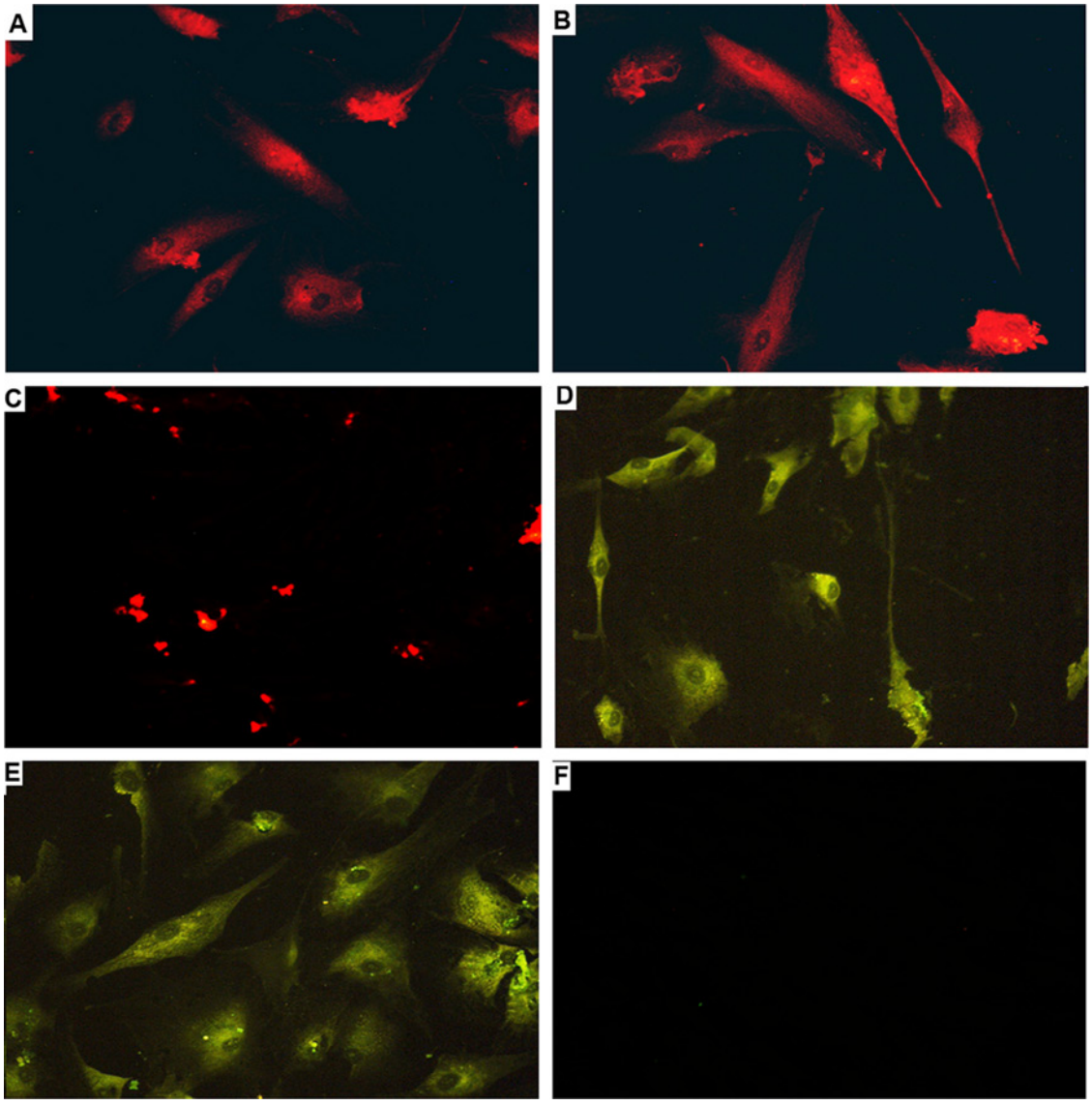

Figure 4 The neural differentiation potential of umbilical cord mesenchymal stem cells (UC-MSCs) was investigated by monoclonal antibodies against human neurofilament M (a), synaptophysin (b), tubulin (d), galactoserebroside (e) and controls (c and f) (fluorescence microcope, Olympus, ×400)

MYST2, *SOX1, GDF2, GDF3, GDF11, RB1, IL17B* and *IL17C* (Table 3). Among these genes, which were expressed at levels at least 100 times lower in UC-MSCs than in the other three cells, were *COL2A1* and *GDF11*.

Discussion

In this study, UC-MSCs were successfully isolated from human UCs. In culture medium containing 20% FBS, the growth and proliferation of UC-MSCs were similar to those of previous reports,[13,15] and there was similar expression of cell surface antigens. The adipogenic, osteogenic, and neural differentiation of UC-MSCs was also effectively induced, as expected. These results indicate that we established a useful method to isolate and culture UC-MSCs for further research or clinical applications.

There have been many reports on the culture of UC-MSCs and their use in cell-based therapy; however, to date, little research has focused on gene expression in these cells, especially on stemness gene expression profiles. Such information is essential for understanding the mechanisms of migration, differentiation and remodeling of UC-MSCs. Although Li *et al.*[16] identified the differential expression of proteins between UC-MSCs and BM-MSCs in a proteomic study, and Feldmann *et al.*[17] and Angelucci *et al.*[15] compiled an expression profile of UC-MSCs by proteome analysis, the basal and unique stemness gene expression profiles of UC-MSCs remain unclear, as precise data about the expression levels of most proteins could not be identified by proteome analysis. In the current study, we assessed expression levels of 168 pathway-focused genes in UC-MSCs, and compared them with those of human normal cell and tumor cells. A significant difference was found between them.

Using realtime PCR, we determined that gene expression levels in UC-MSCs were much higher for some genes, which

Table 2 Genes for which expression levels in UC-MSCs were >10-fold higher than that in KMB17, BEL-7404 and SPC-A-1 cells

UC-MSCs versus					
KMB17		**SPC-A-1**		**BEL-7404**	
Gene	**Fold expression**	**Gene**	**Fold expression**	**Gene**	**Fold expression**
Stem cell-specific markers					
CDC2	54.33 ± 9.06	*CCND1*	158.75 ± 11.86	*APC*	107.01 ± 27.67
FGF1	729.67 ± 45.12	*CDC2*	15.66 ± 1.67	*CCND2*	29.49 ± 3.23
NEUROG2	4996.15 ± 453.21	*HSPA9*	412.17 ± 14.66	*HSPA9*	52.86 ± 2.53
FGF2	33.44 ± 4.05	*BMP2*	49.61 ± 8.58	*BMP2*	11.95 ± 2.45
JAG1	10.77 ± 1.13	*CTNNA1*	51.06 ± 3.6	*JAG1*	15.37 ± 1.45
ALDH2	17.13 ± 5.6	*SOX2*	71.91 ± 11.73	*CD4*	3548.76 ± 456.23
ADAR	34 ± 5			*MYST1*	12.64 ± 0.87
Stem cell differentiation markers					
KRT15	21.79 ± 3.65	*FOXA2*	53.73 ± 11.83	*FOXA2*	3322.61 ± 222.12
ALPI	12.49 ± 1.22	*ISL1*	39.58 ± 5.98	*CD8A*	148.43 ± 11.43
COL1A1	1449476 ± 43212.77	*COL1A1*	82.69 ± 12.01	*COL1A1*	24.79 ± 2.67
CD44	1787.34 ± 231.19	*MME*	12.99 ± 1.86	*MME*	13 ± 1.69
NCAM1	31.69 ± 5.03	*ALP1*	44.34 ± 6.77		
Signaling pathways important for stem cell maintenance					
DTX2	5974.49 ± 987	*DTX2*	11.58 ± 1.83	*DTX2*	12.52 ± 1.56
DVL1	283.87 ± 56			*DLL1*	4930.78 ± 450.12
NUMB	117.1 ± 9.44				

P values are all <0.005

UC-MSC, umbilical cord mesenchymal stem cell; KMB17, human embryonic fibroblast lung diploid cell strain; BEL-7404, human hepatoma cell line; SPC-A-1, human lung adenocarcinama cell line

could be classified into three types: (1) stem cell-specific markers, such as CD44, which is a UC-MSC surface marker, as previously described;[6] (2) stem cell differentiation markers, which include *BMP2*,[18] *JAG1*,[19] *COL1A1*,[20] *DLL1*,[21] *MME* and *KRT15* (bone and cartilage formation),[22] *ALPI* (small intestinal epithelial cell differentiation), *ISL1* (pancreatic cell lineage development),[23] *JAG1*,[24] *NCAM1* (hematopoiesis),[25] *CTNNA1* (epithelial differentiation),[26] *SOX2*,[27] *NCAM1*,[28] *DVL1*,[29] *Neurog2*,[30] *FGF1* and *FGF2* (neuronal differentiation),[31] *ISL1* (pancreatic endocrine markers)[23] and *Numb* (dermis and muscle formation);[32] and (3) signaling pathway proteins important for stem cell maintenance, which include *ALDH2* (aldehyde dehydrogenase family), *FOXA2* (belonging to the forkhead class of DNA-binding proteins, this is a hepatocyte nuclear factor that is a transcriptional activator for liver-specific genes such as albumin and transthyretin), *MYST* (has a chromodomain involved in protein–protein interactions and targeting transcriptional regulators to chromatin), *HSPA9* (member of the heat shock protein 70 family, which plays a role in the control of cell proliferation, and it may also act as a chaperone), *FGF1* and *FGF2* (function as modifiers of endothelial cell migration and proliferation), and *CCND1* and *CCND2* (belong to the highly conserved cyclin family, whose activity is required for cell cycle G1/S transition or G1/S and G2/M transitions).

Of those genes, *COL1A1* and *DTX2* had much higher expression in UC-MSCs than in the other cell types. The *COL1A1* gene encodes the pro-1 chains of type I collagen, which is a fibril-forming collagen found in most connective tissues, and is particularly abundant in bone, cornea, dermis and tendon. This is an osteogenesis-related gene, as gene mutations are associated with disorders such as osteogenesis imperfecta types I–IV,[33] Ehlers-Danlos syndrome type VIIA and the classic type,[34] and Caffey disease and idiopathic osteoporosis.[35]

MSCs can be isolated from several tissues. They can be induced by chondrogenic differentiation medium to differentiate into chondrocyte-like cells *in vitro*,[36] and are thought to be the adult equivalent of the embryogenic cartilage and bone precursor cells. A high expression of mRNA encoding *COL1A1* is found in unstimulated MSCs.[37] Following injection of BM-MSCs into a porcine model of myocardial infarction, increases in *COL1A1* expression were observed in the infarcted hearts of MSC-treated pigs compared with the placebo group.[38] It has been demonstrated that cyclic adenosine monophosphate-mediated protein kinase A (PKA) activation induces *in vitro* osteogenesis and *in vivo* bone formation, with increased levels of the osteogenic marker *COL1A1* in human MSCs.[39]

DTX2 is a gene encoding a WWE-RING-finger protein, and is involved in regulating heart development and heart function. Northern blot analysis revealed that *DTX2* is expressed in 18- and 22.5-week human embryo hearts and in adult hearts, with particularly high levels in 18-week and adult hearts.[40] Members of the DTX (Deltex) family act as Notch signaling modifiers, and may also regulate transcription through interactions with specific transcription factors. The Notch pathway is a conserved signaling mechanism that regulates cellular differentiation in a variety of tissue types throughout the life of multicellular organisms. Notch signals have been shown to influence cell proliferation, apoptosis and developmental lineage choices. They are also essential for promoting several stages of T-cell development[41] and mouse preimplantation development.[42] Although it is well established that Deltex interacts with the intracellular domain of Notch, the mechanism by which Deltex regulates Notch signals, either positively or negatively, remains unclear.

DTX2 functions as an E3 ligase based on its capacity for self-ubiquitination.[43] Previous identification of a Deltex null mutant in *Drosophila* revealed that regulation of Notch through Deltex is strongly cell type-specific.[44] *DTX2*

Table 3 Genes for which expression levels in UC-MSCs were <10-fold lower than that in KMB17, BEL-7404 and SPC-A-1 cells

UC-MSCs versus					
KMB17		SPC-A-1		BEL-7404	
Gene	Fold expression	Gene	Fold expression	Gene	Fold expression
Signal pathway ligands and cytokines					
IFNA5	0.0461 ± 0.0099	*DLL3*	0.011 ± 0.0041	*DLL3*	0.0500 ± 0.0096
TNFSF12	0.0484 ± 0.0211	*DTX1*	0.0178 ± 0.0062	*WNT1*	0.0500 ± 0.0125
IFNK	0.0448 ± 0.0167	*JAG1*	0.013 ± 0.0021	*TERT*	0.0500 ± 0.0181
TNFSF8	0.0239 ± 0.0021	*WNT1*	0.051 ± 0.023	*FIGF*	0.0012 ± 0.00033
INHBA	0.0638 ± 0.0185	*FIGF*	0.00038 ± 0.00010	*TNFSF11*	0.0021 ± 0.00058
GDF11	0.0093 ± 0.0012	*TNFSF11*	0.00057 ± 0.00015	*GDF11*	0.0030 ± 0.00038
GDF9	0.0942 ± 0.0187	*GDF11*	0.00898 ± 0.00114	*TNFSF14*	0.020 ± 0.0019
KAT2A	0.097 ± 0.0094	*BMP8B*	0.02738 ± 0.00700	*BMP5*	0.031 ± 0.0046
SIGMAR1	0.098 ± 0.002	*IFNK*	0.03680 ± 0.01375	*TNFSF8*	0.021 ± 0.0019
CD70	0.0530 ± 0.0183	*TXLNA*	0.05218 ± 0.01331	*IFNA1*	0.078 ± 0.0257
TXLNA	0.0575 ± 0.0147	*IFNB1*	0.07316 ± 0.01581	*TNFSF13*	0.060 ± 0.0125
IFNA2	0.0584 ± 0.0217	*IFNA4*	0.08374 ± 0.02898	*IL2*	0.0027 ± 0.00064
CCDN1	0.078 ± 0.019	*IFNA1*	0.09399 ± 0.03097	*IL17C*	0.0030 ± 0.00030
CSF1	0.0198 ± 0.0016	*LTA*	0.09994 ± 0.02083	*IL17B*	0.0066 ± 0.00092
IFNA4	0.0209 ± 0.0072	*IL2*	0.00108 ± 0.00025	*IL1A*	0.018 ± 0.0024
IL1F10	0.0487 ± 0.0153	*IL5*	0.00695 ± 0.00126	*IL4*	0.021 ± 0.0037
IL17C	0.0426 ± 0.0043	*IL17B*	0.01940 ± 0.00274	*IL5*	0.087 ± 0.0157
IL1F5	0.0331 ± 0.0068	*IL17C*	0.02168 ± 0.00217	*IL3*	0.059 ± 0.0181
IL17A	0.0457 ± 0.0149	*IL24*	0.03432 ± 0.00527	*IL1F7*	0.062 ± 0.0066
IL1A	0.0243 ± 0.0034	*IL19*	0.07433 ± 0.01727	*IL1F6*	0.058 ± 0.0145
IL21	0.0637 ± 0.0238	*IL1F7*	0.09006 ± 0.00965		
IL15	0.0647 ± 0.0163	*IL1B*	0.09906 ± 0.01429		
IL17B	0.0082 ± 0.0012				
IL25	0.0499 ± 0.0123				
IL13	0.0509 ± 0.0181				
IL16	0.0561 ± 0.0103				
IL9	0.0629 ± 0.0206				
TNF	0.0351 ± 0.0120				
Genes associated with cell growth and differentiation					
CDC42	0.048 ± 0.015	*FGF3*	0.0234 ± 0.0086	*EP300*	0.0071 ± 0.0020
HDAC2	0.042 ± 0.0082	*MYST2*	0.0289 ± 0.0082	*MYST2*	0.0600 ± 0.0266
MYST2	0.017 ± 0.0032	*SOX1*	0.0267 ± 0.0067	*SOX1*	0.0500 ± 0.0145
SOX1	0.0033 ± 0.0007	*BMP3*	0.032 ± 0.012	*CXCL12*	0.0500 ± 0.0186
BMP1	0.0934 ± 0.0216	*GDF2*	0.012 ± 0.0051	*GDF2*	0.0200 ± 0.0140
CXCL12	0.03 ± 0.0072	*GDF3*	0.05525 ± 0.01111	*GDF3*	0.0200 ± 0.0080
CSF2	0.0651 ± 0.0263	*GJB1*	0.053 ± 0.017	*GJB1*	0.0700 ± 0.0257
GJB1	0.021 ± 0.0052	*NCAM1*	*0.058 ± 0.024*	*ACTC1*	0.0200 ± 0.0300
CD4	0.035 ± 0.0091	*T*	0.0164 ± 0.0054	*MSX1*	0.0200 ± 0.0037
ABCG2	0.069 ± 0.023	*CD8B*	0.027 ± 0.0091	*T*	0.0900 ± 0.0188
IFNG	0.0391 ± 0.0121	*ACAN*	0.082 ± 0.051	*ACAN*	0.0300 ± 0.0080
TGFB1	0.0305 ± 0.0099	*COL2A1*	0.00037 ± 0.00015	*COL2A1*	0.0091 ± 0.0027
GDF2	0.0046 ± 0.00095	*TERT*	0.056 ± 0.034		
GDF3	0.0069 ± 0.0011	*IFNG*	0.02831 ± 0.00876		
ACTC1	0.0077 ± 0.0014	*TGFB1*	0.06077 ± 0.01975		
ASCL2	0.049 ± 0.01				
PDX1	0.049 ± 0.011				
COL2A1	0.0013 ± 0.00052				
ISL1	0.025 ± 0.0063				
Tumor suppressor or related genes					
RB1	0.008 ± 0.0018	*RB1*	0.0178 ± 0.0061	*RB1*	0.0400 ± 0.0037
CCNE1	0.024 ± 0.0081	*CCNE1*	0.0302 ± 0.0072	*CCNE1*	0.0900 ± 0.0357
CDH1	0.022 ± 0.0062	*CDH1*	0.034 ± 0.0056	*CDH1*	0.0900 ± 0.0224
APC	0.022 ± 0.0073				

P values are all <0.005

UC-MSC, umbilical cord mesenchymal stem cell; KMB17, human embryonic fibroblast lung diploid cell strain; BEL-7404, human hepatoma cell line; SPC-A-1, human lung adenocarcinama cell line

transcripts are detected in all embryonic stages during mouse preimplantation development.[42] In the present study, *DTX2* was strongly expressed in UC-MSCs, where it may regulate Notch signals and cellular differentiation. Clarification of its functions requires further research.

In contrast, several genes had lower expression in UC-MSCs than in the other three cell types. Of these genes, *CCNE1*, *CDH1*, *COL2A1*, *MYST2*, *SOX1, GDF2, GDF3, GDF11, RB1, IL17B* and *IL17*C had 10-fold lower expression levels, while *COL2A1 and GDF11* had 100-fold lower expression in UC-MSCs than in the other cell types. *CCNE1* belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Overexpression

of this gene, which results in chromosome instability, has been observed in many tumors, and thus may contribute to tumorigenesis.[45,46] *MYST2* regulates gene expression by intimately connecting to post-translational modification of histones bound to DNA. It has central and specific roles in regulating cellular processes ranging from apoptosis, cell cycle and adult stem cell homeostasis, to patterning of the early embryo.[47] *RB1* is a negative regulator of the cell cycle and was the first tumor suppressor gene found. The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure.[48–50] *SOX1* encodes a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and determination of cell fate. In mice, a similar protein regulates the gamma-crystallin genes and is essential for lens development.[51] *IL17B* shares structural similarities to *IL17A*, which has been proposed to promote angiogenesis and tumorigenicity. This cytokine in articular cartilage has important implications for cartilage homoeostasis and regeneration.[52,53] *IL17C* was reported to stimulate the release of TNF alpha (TNF-α) and IL1 beta (IL-1B) from a monocytic cell line. The expression of this cytokine was found to be restricted to activated T-cells.[54]

Interestingly, the members of the *COL1A1*/*COL2A1* families had different expression levels in UC-MSCs. *COL2A1* encodes the alpha-1 chain of type II collagen, which is the main component of the extracellular matrix of articular cartilage. Mutations in this gene are associated with achondrogenesis, chondrodysplasia, early-onset familial osteoarthritis, spondyloepiphyseal dysplasia congenita, Langer-Saldino achondrogenesis, Kniest dysplasia, Stickler syndrome type I and spondyloepimetaphyseal dysplasia Strudwick type.[55] *GDF11* is a member of the bone morphogenetic protein (BMP) family and the TGF-beta superfamily. Studies in mice and xenopus suggest that this protein is involved in mesodermal formation and neurogenesis during embryonic development.[56] Our results showed that *COL2A1* and *GDF11* expression was significantly downregulated in UC-MSCs, while upregulated in the other three cells. Further studies will be necessary to determine the reason for this expression pattern.

Although some reports suggest that MSCs can suppress tumor growth in prostate cancer,[57] syngeneic glioma[58] and breast cancer,[59] other reports indicate that MSCs can promote tumor engraftment and metastatic colonization in a rat osteosarcoma model,[60] and can secrete soluble signaling molecules that potentiate tumor growth.[61] However, there are no reports with regard to whether MSCs themselves can turn into tumor cells. In our study, it is noteworthy that some tumor-related or tumor suppressor genes, such as *RB1*, *CDH1* and *APC*, had a lower expression in UC-MSCs than in the other three cell types, suggesting that UC-MSCs are quite different from tumor cells in mRNA expression profile, and this provides some insights into the fact that UC-MSCs do not easily change into tumor cells.

Taken together, our results contribute to the understanding of how UC-MSCs can be defined in terms of their gene expression, and also provide evidence that some proteins might be linked to functional stem cell capacities such as self-renewal and commitment. Further studies will be necessary to derive the underlying molecular mechanisms. This is the first report on the pathway-focused protein profile of UC-MSCs and other cells, which could provide an insight into the use of UC-MSCs as a promising source for cell therapy. It may also assist in the identification of functional expression profiles in human MSCs of UC origin.

Author contributions: Z-LH conceived and designed the experiments; Z-LH and M-YM collected the samples; WP and L-HJ carried out analysis of the data and review of the manuscript; M-YM, Y-HL, C-YW, Y-HX and H-DY conducted the experiments; and WP and M-YM wrote the manuscript. M-YM and WP contributed equally to this work.

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