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Comparison of phenotypes and transcriptomes of mouse skin-derived precursors and dermal mesenchymal stem cells

Yiming Li[a,b,1], Xiaohua Li[a,1], Lidan Xiong[a], Jie Tang[a], Li Li[a,*]

[a] Department of Dermatology and Venerology, Huaxi Hospital, Chengdu, Sichuan Province, PR China
[b] Department of dermatology and venerology, the First Affiliated Hospital of Chengdu Medical College, 278 Baoguang Ave, Xindu district, Chengdu, Sichuan Province, PR China

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ABSTRACT

Both skin-derived precursors (SKPs) and dermal mesenchymal stem cells (dMSCs) are promising candidates for cellular therapy and regenerative medicine. To date the comparison of phenotypes and transcriptomes of mouse SKPs (mSKPs) and dMSCs has never been reported. Here we characterized and compared the biological properties and transcriptomes of mSKP and dMSCs from the same mouse dermis sample. Firstly, we analyzed mSKPs and dMSCs by use of immunocytochemistry, cell cycle analysis, and CD antigen expression. Then we conducted the osteogenic, adipogenic, and chondrogenic induced differentiation for both cell types. Lastly, we compared their genomic profiles by RNA-sequencing (RNA-Seq), and verified the results of RNA-Seq by quantitative real time reverse transcription PCR (qRT-PCR). The results suggested that mSKPs and dMSCs shared similarities in certain positive stem cells markers expression, but demonstrated difference in Nanog and Oct4 expression. mSKPs and dMSCs demonstrated similar cell cycle distribution and CD antigen expression. Both types of cells could be induced differentiated into osteocytes, adipocytes, and chondrocytes. However, RNA-Seq and qRT-PCR results indicated that mSKPs and dMSCs had distinct transcriptome profiles. The majority of enriched differentially expressed genes (DEGs) from mSKPs was immune-related, while the majority of enriched DEGs from dMSCs was differentiation/development/disease-related. Transcriptome profiles suggested that mSKPs and dMSCs might have potential usage in the relevant morbidity management. These results may indicate a molecular basis for novel stem cell-based therapeutic strategies.

1. Introduction

Skin-derived precursors (SKPs) and dermal mesenchymal stem cells (dMSCs) from dermis, with different morphology and growth pattern, both possess the capacities of self-renewal and multipotency. Developments in stem cell research have generated much excitement about the potential for regenerative medicine and cell-based therapies.

Under proper inducing conditions, SKPs can differentiate into hepatic cells (De Kock et al., 2009), osteoblasts (Suriyachand et al., 2011), corneal epithelial-like cells (Saichanma et al., 2012), vascular smooth muscle cells (Steinbach et al., 2011), neurons and Schwann cells (Sparling et al., 2015; Kumar et al., 2016; Gingras et al., 2007; Krause et al., 2014; Shakhbazau et al., 2014; Khuong et al., 2014a), fibroblast-like cells (Shu et al., 2014), enteric-type neurons (Kwok et al., 2013; Wagner et al., 2014a, b), and islet-like insulin-producing cells (Mehrabi et al., 2015). The capacity of generating functional neurons, glia, and mesenchymal cells has enabled SKPs a promising candidate for regenerative medicine and cell transplantation (Hunt et al., 2009). SKPs could also serve as an in vitro modelling tool for the study of diseases, such as type 1 neurofibromatosis (Gutiérrez-Rivera et al., 2012).

MSCs' ability to differentiate across various lineages beyond the mesodermal lineages has suggested a useful therapeutic approach for various disorders. MSCs have been reported to be employed in multiple clinical scenario, such as chronic lung diseases (Antoniou et al., 2018), multiple sclerosis (Riordan et al., 2018), aplastic anemia (Xu et al., 2018), liver diseases and inflammatory bowel diseases (Tsuchiya et al., 2017), bone defects (Killington et al., 2017), cardiovascular regeneration (Majka et al., 2017), and idiopathic pulmonary fibrosis (Glassberg et al., 2017). The immunosuppressive and/or immunomodulatory properties have contributed to their application in regenerative medicine and tissue repair (Kim and Cho, 2013).

To date, no in-depth comparative study, especially the one covering

* Correspondence to: Department of dermatology and venerology, Huaxi Hospital, 37 Guoxue Alley, Wuhou district, Chengdu, Sichuan Province 610041, PR China.
E-mail address: cosmeticphysician@163.com (L. Li).
[1] Yiming Li and Xiaohua Li contributed equally to this manuscript.

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the transcriptomes, has been reported. In the present study, we compared mouse SKPs (mSKPs) and dMSCs from the same mouse dermis sample by immunocytochemistry, cell cycle analysis, CD antigen expression, and induced differentiation. We also investigated the possible molecular mechanisms underlying their similarities and diversities by use of RNA sequencing (RNA-Seq). Deciphering the unannotated transcriptional activity and identifying numerous novel transcripts might provide further insight into stem cell growth pattern, differentiation, and most importantly, the potential therapeutic usage.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Animal Ethics Committee of Huaxi hospital, Sichuan University (Approval No. 2017064 A) and was strictly carried out under the guide for the care and use of laboratory animals.

2.2. Culture medium setup

mSKPs culture medium: DMEM/F12 (3:1, Invitrogen, USA) containing 0.1% penicillin/streptomycin (Invitrogen, USA), 40 ng/mL bFGF (Millipore, USA), 20 ng/mL EGF (Millipore, USA), and 2% B27 supplement (Gibco, USA).

dMSCs culture medium/basal medium for dMSCs: low glucose DMEM (Invitrogen, USA) containing 10% FBS (Clarks, Australia) and 1% penicillin/streptomycin.

basal medium for mSKPs: mSKPs culture medium containing 5% FBS.

2.3. Cell isolation and culture

Starting with standard culture protocols previously reported in the literature (Biernaskie et al., 2006) and conducted in our lab (Mao et al., 2015), we isolated the cell suspension from neonatal Balb/C mice (aged 1–3 day) dermis. At the final step, different protocols were employed for mSKPs and dMSCs culture, respectively. For mSKPs culture, the plating density was 2.5×10^5 cells/mL, and the volume of mSKPs culture medium was 5 mL in a 25 cm^2 flask. Cultures were fed every 3 days with an addition of 1 mL fresh medium containing all growth factors and supplements (bFGF, EGF, B27) at a concentration that would replenish the entirety of the culture medium. On day 10, the mSKPs were collected for the following experiments. For dMSCs culture, the plating density was 2.5×10^5 cells/mL, and the volume of dMSCs culture medium was 10 mL in a 10 cm^2 dish. The medium was replaced with the fresh medium every 3 days, and the cells were passaged when the confluency reached 80–90%.

2.4. Immunocytochemistry

Cells were plated on slides, fixed by 4% paraformaldehyde. The fixed cells were blocked with 3% BSA for 30 min and subsequently incubated with primary antibody overnight at 4 °C. After being washed with PBS for 3 times, cells were incubated with secondary antibody for 1 h at room temperature. Finally cells were incubated with DAPI (Dogindo, Japan) for 1 min. Primary antibodies were anti-α-SMA (Abcam, UK, 1:500), anti-Nanog (Abcam, UK, 1:250), anti-Oct4 (Abcam, UK, 1:250), anti-Pan Cytokeratin (Boster, Wuhan, 1:1000), anti-Ssea 4 (Abcam, UK, 1:250), anti-Versican (Boster, Wuhan, 1:250), anti-Vwf (Boster, Wuhan, 1:500), anti-Fibronectin (Abcam, UK, 1:250), anti-Vimentin (Abcam, UK, 1:200), anti-Nestin (Abcam, UK, 1:500), anti-Sox2 (Boster, Wuhan, 1:250), and anti-Collagen I (Abcam, UK, 1:500). Secondary antibodies were Alexa Fluor® 488 donkey anti-mouse (Abcam, UK, 1:500) and Alexa Fluor® 594 goat anti-rabbit (Abcam, UK, 1:500). The protocol was performed in triplicate for both cell types.

Table 1
Primer sequences for qRT-PCR.

No.	Gene	Primer sequences
Internal reference	ACTIN-F	CACCCGCGAGTACAACCTTC
	ACTIN-R	CCCATACCCACCATCACACC
1	A2m-F	GGAGGAGTAGAAGATGAAG
	A2m-R	ATGTGGTTCTGGTAATCA
2	Cx3cr1-F	CCTGCCCTTGCTTATCAT
	Cx3cr1-R	GCCTTCTTGCGATTCTTG
3	Tnf-F	TTCTCATTCCTGCTTGTG
	Tnf-R	AACTTCTCATCCCTTTGG
4	Vav1-F	ATATGTTCCTTCTGATTGAG
	Vav1-R	TTCCATCCACTTCTTCTT
5	Myh11-F	AAGATGATGAGATGTTCCA
	Myh11-R	AGATGATACTACCTTCAAGAT
6	Ctgf-F	CCAATGACAATACCTTCT
	Ctgf-R	TTTGCCCTTCTTAATGTT
7	Actg2-F	CCAAAGCAAACAGAGAGAA
	Actg2-R	AGCCTGAATAGCAACATAC
8	Itgb4 -F	CAATACAAGACACAGGAC
	Itgb4 -R	TAAGAGTAGTTGGTGACA

2.5. Cell cycle analysis

10^6 cells were harvested and washed in 1 mL cold PBS, then centrifuged at 2000 rpm for 5 min at 4 °C. 0.5 mL 70% cold ethanol was added drop-wisely to fix the cells after the supernatant was discarded. Cells were left at 4 °C overnight, and washed twice in cold PBS. 100 μL RNAase (KeyGEN BioTECH, Nanjing) was added to each sample. Samples were then incubated in the 37 °C water bath for 1 h before 400 μL PI solution (KeyGEN BioTECH, Nanjing) was added. Samples were kept in darkness at 4 °C before being analyzed on CytoFLEX flow cytometry system (Beckman Coulter, USA) by reading on cytometer at 488 nm.

2.6. CD antigen expression

10^7 cells were collected, re-suspended in 1 mL cold PBS containing 1% FBS, and centrifuged at 300 RCF for 5 min at 4 °C. Cells were transferred to another EP tube, CD antibodies (BD Pharmingen, USA) were added to the tubes and incubated in darkness in crushed ice for 30 min 200 μL cold PBS containing 1% FBS was added to each tube, and cells were analyzed on CytoFLEX flow cytometry system (Beckman Coulter, USA). The volume of antibodies was 2 μL (anti-CD14, anti-CD45, anti-CD11b, anti-CD34) or 5 μL (anti-CD105, anti-CD73, anti-CD90.2), respectively.

2.7. Osteogenic, adipogenic, and chondrogenic induced differentiation

For osteogenic or adipogenic induced differentiation, cells were trypsinized, dissociated into single cells, and then re-suspended and cultured in basal medium. The basal medium was replaced by osteogenic or adipogenic induced differentiation medium provided in the induced differentiation kits (Cyagen, USA) when the confluency reached 60–70%. The following steps were consistent with the protocol within the kits. At the end of a 28-day induced differentiation, cells were stained with Alizarin Red Solution (for osteocytes induced differentiation) or Oil Red Solution (for adipocytes induced differentiation) provided in the kits, respectively.

For chondrogenic induced differentiation, cells were collected and induced differentiated according to the protocol within the chondrogenic differentiation kit (Cyagen, USA). The chondrogenic pellets were harvested after 30 days in the 15 mL tubes culture. Pellets were 10% formalin fixed, paraffin embedded, machine sliced, and then stained with Alcian blue (Leagene biotechnology, Beijing) according to the protocol.