Integrated analysis of DNA methylome and transcriptome reveals the differences in biological characteristics of porcine mesenchymal stem cells from bone marrow and umbilical cord

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Abstract

Background Bone marrow (BM) and umbilical cord (UC) are the main sources of mesenchymal stem cells (MSCs). These two MSCs display significant differences in many biological characteristics, yet the underlying molecular mechanisms need to be explored. **Results** In this study, to better understanding the biological features of MSCs, we isolated BMMSCs and UCMSCs from inbred Wuzhishan miniature pigs and generated the first global DNA methylation and gene expression profiles of porcine MSCs. The results showed that the osteogenic and adipogenic differentiation ability of porcine BMMSCs is stronger than that of UCMSCs. Stem cell surface marker CD90 were positively detected in both BMMSCs and UCMSCs. 587 genes were differentially methylated (280 hypermethylated and 307 hypomethylated) at the promoter regions between BMMSCs and UCMSCs. Meanwhile, 1,979 differentially expressed genes (1,407 up-regulated and 572 down-regulated) were identified between BMMSCs and UCMSCs. Integrative analysis reveals that 120 genes displayed differences in both gene expression and promoter methylation. Gene Ontology enrichment analysis revealed that these differential genes were associated with cell differentiation, cell migration, and immunogenicity properties. Remarkably, skeletal system development related genes were significantly hypomethylated and up-regulated in UCMSCs, while cell cycle genes were significantly higher down-regulated and hypermethylated, implying UCMSCs have higher cell proliferative activity and lower osteogenic differentiation potential than BMMSCs. **Conclusions** Our results indicate that DNA methylation plays an important role in regulating the biological characteristics differences between BMMSCs and UCMSCs. The study might provide a molecular theory basis for the application of porcine MSCs in human.

Background

Mesenchymal stem cells (MSCs), also known as seed cells, are widely used in tissue repair and regeneration because of their self-renewal and differentiation capacity together important immunosuppressive properties and low immunogenicity ([Kolf et al., 2007](#); [Shi et al., 2010](#); [Li and Hua, 2017](#)). MSCs were originally isolated from bone marrow (BM). However, the use of BMMSCs is not always acceptable due to the highly invasive donation procedure and the significant decline in cell number and proliferative/differentiation capacity with age ([Romanov et al., 2003](#)). In recent years, studies have discovered other MSC sources from almost every tissue of the body, such as adult adipose (AT), placenta and amniotic fluid ([Semenov et al., 2010](#); [Ivana et al., 2011](#); [Urrutia et al., 2019](#)). Additionally, umbilical cord MSCs (UCMSCs) have been introduced as an promising source of MSCs and applied in preliminary clinical treatments, because they can be easily obtained, display less negative effects on the donor and avoid certain ethical questions ([Lindenmair et al., 2012](#); [Chandravanshi and Bhonde, 2018](#)). Though MSCs derived from different sources share many similar biological characteristics, they also exhibit distinct and unique gene expression and functional properties ([Si et al., 2011](#); [Cho et al., 2017](#)).

The miniature pig (*Sus scrofa*) is an attractive and realistic large animal model, because of the anatomical, physiological and genomic similarities to human ([Vodička et al., 2010](#); [Wang et al., 2010](#)). Over 25 years, the inbred Wuzhishan miniature pig was developed by the Institute of Animal Sciences,

Chinese Academy of Agricultural Sciences. The inbred WZSP line has high genetic stability (Fang et al., 2012) and the inbreeding coefficient at the twenty-fourth generations reached 0.994 in 2013 (Mu et al., 2015), which has been widely used for studying human diseases, including atherosclerosis, cardiovascular disease, xenotransplantation, and diabetes (Dong et al., 2014; Zhao et al., 2018). Due to the source and availability of a large quality of human MSCs is limited, the therapeutic potentialities of MSCs derived from animal sources have acquired wide attention (Bai et al., 2012; Lu et al., 2014; Khatri and Richardson, 2017). Porcine MSCs can be easily obtained and share similar morphology and multilineage differentiation potential to those of human MSCs (Groth et al., 2012). MSCs derived from inbred WZSP are highly stable and conducive to establish a reliable system to evaluate the biological characteristics of porcine MSCs.

DNA methylation is a stable epigenetic modification that regulates many biological processes, including genomic imprinting, X-inactivation, genome stability and gene regulation (Ambrosi et al., 2017). However, there is limited information about the DNA methylation and gene expression regulation of porcine MSCs. In this study, to reveal the molecular mechanism of the biological characteristic differences of MSCs, we isolated BMMSCs and UCMSCs from inbred WZSP. The genome-wide DNA methylome and transcriptome maps of BMMSCs and UCMSCs were generated by methylated DNA immunoprecipitation sequencing (MeDIP-Seq) and RNA sequencing (RNA-seq), respectively. We identified a set of genes displaying expression and methylation differences between these two MSCs, which are critical for regulating the biological functions of porcine MSCs. This study provides a molecular theory basis of the application of porcine MSCs in clinical therapy.

Methods

Isolation and culture of porcine mesenchymal stem cells

The WZSP littermates were purchased from the National Germplasm Resources Center of Laboratory Miniature Pig, Beijing, China. All animal procedures were approved by the Animal Care and Use Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. The pigs were injected intravenously with propofol (2 mg/kg) to induce full anesthesia. UCMSCs were isolated from the umbilical cord of four WZSP littermates on the day of birth, and BMMSCs were isolated from the bone marrow of the same individuals on 42 days after birth. To isolate BMMSCs, the bone marrow was extracted and centrifuged for 5 min at 1000 rpm. To isolate UCMSCs, the umbilical cords were cut into 1-2 mm² pieces, attached and cultured. The isolated MSCs were cultured in DMEM/F12 medium (Gibco) with 20 % fetal bovine serum (Gibco), 50 units/ml penicillin G, and 50 µg/ml streptomycin and incubated at 37°C in 5% CO₂ in a humidified incubator; the medium was replaced every 3 days.

Flow cytometric analysis of cell surface antigen expression

Flow cytometry was used to analyze the surface marker phenotype of the MSCs, similar to our previous report. Cells were harvested by 0.05 % trypsin-EDTA for 3 minutes at 37 °C, followed by washing and fixation. MSCs were resuspended in 1% (w/v) bovine serum albumin (Sigma) for 30 min in room temperature to block the non-specific binding sites. After blocking, the BMMSCs were incubated with CD29 (VMRD), CD44 (VMRD), CD45 (VMRD) and FITC-anti-human CD34/PE-anti human CD90 (eBioscience) monoclonal antibodies at room temperature for 20 min. The UCMSCs were incubated with CD31, CD45 (Veterinary Medical Research & Development, VMRD) and FITC-anti-human CD34/PE-anti human CD90 (eBioscience) monoclonal antibodies, respectively, at room temperature for 20 min. The CD29, CD44 and CD45 groups were then stained with rat anti-mouse IgG1-FITC (IVGN), goat anti-mouse IgG2a-PE secondary antibody (IVGN) and anti-mouse IgM-PE (eBioscience) at room temperature for 20 min, respectively. Flow cytometric acquisition and data analysis were performed with BD FACS Calibur Flow Cytometer and Cell Quest software. As a negative control, the cells were incubated only with DPBS. Each flow cytometry experiment was performed in triplicate.

Adipogenic and osteogenic differentiation of porcine BMMSCs and UCMSCs

To evaluate the differentiation ability of MSCs in vitro, we replaced the DMEM/F12 medium with an adipogenic/osteogenic differentiating medium when cells reached 80 % confluency. Cells were cultured for 2 or 3 weeks before collection and the medium were changed every 3 days. After 2 or 3 weeks, Oil red O was used in the analysis of adipogenic differentiation and alizarin red staining was used in the analysis of osteogenic differentiation.

Methylated DNA immunoprecipitation sequencing

Genomic DNA was isolated using E.Z.N.A. HP Tissue DNA Midi Kit (Omega) and was sonicated to 100-500 bp fragments with a Bioruptor Sonicator (Diagenode). Four BMMSCs DNA samples were pooled and four UCMSCs DNA samples were pooled by homogeneous mixing prior to methylated DNA immunoprecipitation sequencing. The libraries were constructed following the manufacturer's instructions, same to our previous reports ([Yang et al., 2016](#); [Yang et al., 2017](#)), and sequenced on an Illumina HiSeq 2000 with 49 bp paired-end reads.

MeDIP-seq data analysis

After filtering the low-quality reads that contained more than 5 'N's or over 50 % of the sequence with low quality value (Phred score < 5), the clean reads were aligned to the pig reference genome downloaded from the USCS database, allowing up to two mismatches using SOAP2 (v2.21) ([Li et al., 2009](#)). Reads mapping to the same genomic location were regarded as potential clonal duplicates due to PCR

amplification biases. To avoid stochastic sampling drift, we filtered out CpG sites that were covered by less than a 10 read depth (Li et al., 2012). Model-based Analysis of ChIP-Seq (MACS v1.4.2) (<http://liulab.dfci.harvard.edu/MACS/>) was used to scan the methylated peaks in pig genome with default parameters (Zhang et al., 2008). The methylation level of each peak was calculated using the RPKM method. Differentially methylated regions (DMRs) were identified using the exact test for negative binomial distribution with a significance threshold of $FDR < 0.001$ and $|\log_2 FC| \geq 1$. We defined the region 2 kb upstream of TSS as promoter and the region from the TSS to TTS as gene body. The promoters that contained one or more DMRs were considered as differentially methylated promoters for further analysis.

Transcriptome sequencing and data analysis

RNA from BMMSCs and UCMSCs was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA), treated with DNase I (Qiagen) and cleaned using the RNAeasy MiniElute Cleanup kit (Qiagen, Basel, Switzerland). The integrity of total RNA was checked by an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and only RNA samples with a RNA Integrity Number (RIN) score > 8 were used for RNA sequencing. Equal amounts of RNA from four samples of BMMSCs and UCMSCs were combined into a pool, respectively. Beads with oligo (dT) were used to isolate poly (A) mRNA after total RNA was collected. Fragmentation buffer was added to fragment the mRNA. Taking these short fragments as templates, a random hexamer primer was used to synthesize the first-strand cDNA. The second-strand cDNA was synthesized using buffer, dNTPs, RNaseH and DNA polymerase I, respectively. Short fragments were purified with QiaQuick PCR extraction kit and resolved with EB buffer for end reparation and adding poly (A). The short fragments were then connected with sequencing adaptors. And, for amplification with PCR, we selected suitable fragments, as templates, with respect to the result of agarose gel electrophoresis. The libraries were sequenced using Illumina HiSeq 2000 to generate 90 bp paired-end reads.

After trimming the adaptor sequences and removing low-quality reads, clean reads were mapped to *Sus scrofa* reference genome using SOAP2 (v2.21) allowing up to three mismatches (Li et al., 2009). RPKM value was used to measure the expression level of each gene. Differentially expressed genes between BMMSCs and UCMSCs were identified using the exact test for negative binomial distribution. Genes with $FDR < 0.001$ and $|\log_2 FC| \geq 1$ were considered as differentially expressed.

GO enrichment analysis

Functional enrichment analysis was performed using the DAVID (Database for Annotation, Visualization and Integrated Discovery) web server (<http://david.abcc.ncifcrf.gov/>) (Da et al., 2009). Genes with DMRs

in promoters were mapped to their human orthologs and were submitted to DAVID for GO enrichment analysis.

RT-qPCR

Total RNA was extracted using the RNA Extraction Kit (BioTeke). First Strand cDNA was synthesized using the oligo (dT)18 primer provided in the RevertAid First Strand cDNA synthesis kit (Thermo). q-PCR was performed on an ABI 7500 machine using the SYBR Premix Ex Taq kit (TaKaRa) and the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as endogenous control gene. Relative expression levels of objective mRNAs were calculated using the $2^{-\Delta\Delta C_t}$ method. Primer sequences are shown in Additional File 1: Table S4.

Sequenom MassARRAY quantitative methylation analysis.

The DNA isolated from UCMSCs and BMMSCs were treated with sodium bisulfite using an EZ DNA Methylation-Gold Kit (ZYMO Research) according to the manufacturer's instructions. Quantitative methylation analysis of the DMRs was performed using the Sequenom MassARRAY platform (CapitalBio, Beijing, China) (Mathias et al., 2005). Specific primers were designed using the EpiDesigner software (Sequenom). The quantitative results for each CpG or multiple CpGs were analyzed with EpiTyper software v1.0 (Sequenom). The primer sequences are shown in Additional File 1: Table S4.

Results

Isolation and identification of porcine BMMSCs and UCMSCs

We isolated the BMMSCs and UCMSCs from the inbred WZSPs. Adhesion of BMMSCs to the plastic flask was observed at 24h after isolation. As the process continued, adherent cells displayed a scatter distribution and grew in isolated clones. UCMSCs gradually grew outward from the umbilical cord tissues after 7 days. The cell morphology of UCMSCs was similar to BMMSCs: The majority of the cells were fusiform and their nucleoli were clear. The passage cells reached 90 % confluency after approximately 3 days (Figure 1A).

Flow cytometry (FCM) analysis was performed to confirm the surface marker characteristics of MSCs. In BMMSCs and UCMSCs, the stem cell surface markers (CD29, CD44 and CD90) were positively detected, whereas leucocyte marker CD45 and hematopoietic lineage marker CD34 were negative (Figure 1B). The UCMSCs were positive for CD90, but negative for CD34, CD45 and endothelial marker CD31 (Figure 1C). The *in vitro* differentiation potential of BMMSCs and UCMSCs for osteogenic and adipogenic lineages were further detected. We observed that the calcified nodules on the cell surface of MSCs increased with the induction of osteoblast differentiation. On the 21st day of osteogenic induction, the morphology of the

cells significantly changed by displaying substantial accumulation of orange sediment (Figure 1D), the calcified nodules of BMMSCs were much more obvious than UCMSCs. On the 21st day after adipogenic differentiation induction, numerous intracellular lipid droplets were formed (Figure 1E), and the lipid droplets of BMMSCs were much more obvious than UCMSCs. The results showed that both MSCs we obtained have the potentials for osteogenic and adipogenic differentiation, while the differentiation ability of BMMSCs is stronger than that of UCMSCs.

DNA methylomes and transcriptome profiling of porcine BMMSCs and UCMSCs

We carried out MeDIP-seq and RNA-seq to profile the genome-wide DNA methylome and transcriptome of porcine BMMSCs and UCMSCs. Approximately 7.2 Gb clean reads were generated for each MeDIP-seq library. 75.52 % and 76.42 % of the reads from the BMMSCs and UCMSCs could mapped to the pig reference genome, respectively. Meanwhile, about 4.8 Gb clean reads were obtained for each RNA-seq library. The clean reads from the BMMSCs and UCMSCs samples aligned to 59.90 % and 59.83 % of the pig reference genome, respectively. After removing duplicated reads, the remaining uniquely aligned reads were used for the following analyses.

Methylome characteristics of the porcine BMMSCs and UCMSCs

We first analyzed the genome-wide DNA methylation pattern of porcine MSCs (Figure 2) and found that the methylation level was negatively correlated with repeat length (Pearson's $r = -0.248$, $p < 0.001$), and positively correlated with gene number (Pearson's $r = 0.335$, $p < 0.001$), CpG islands (CGIs) length (Pearson's $r = 0.482$, $p < 0.001$), CpG sites number (Pearson's $r = 0.777$, $p < 0.001$) and especially with observed over expected number of CpG (CpGo/e) ratio (Pearson's $r = 0.790$, $p < 0.001$). We further analyzed the methylation of the upstream 2 kb regions of the transcription start sites (TSS), the gene body and the downstream 2 kb regions of the transcription termination site (TTS) for MSCs (Figure 3). The TSS of both MSCs displayed low methylation, while the DNA methylation level of gene body regions was relatively constant, which was much higher than the 5' and 3' flanking regions. These results were consistent with previous reports (Yang et al., 2016; Yang et al., 2017).

Promoter methylation and transcriptional repression in MSCs

Methylated peaks were detected across different genomic elements. Reads per Kilo bases per million reads (RPKM) value was used to evaluate the methylation level of each peak. A total of 150,690 and 161,105 methylated peaks were generated with average lengths of 1,462 and 1,466 bp in BMMSCs and UCMSCs, respectively, covering 9.74 % and 10.44 % of the *Sus scrofa* genome. We classified genes into four groups according their methylation modification: (I) only promoter was modified; (II) only genebody

was modified; (III) both were modified; (IV) neither promoter nor genebody were modified. The number of genes classified into these four methylation types in BMMSCs was 1,134, 8,424, 2,213 and 8,656, respectively (Figure 4A); and the number in UCMSCs was 1,187, 8,106, 2,520 and 8,614, respectively (Figure 4B). The expression level of genes in group IV was significantly higher than that of genes in other three groups, while the genes in group I exhibited the lowest expression levels (Figure 4C). These results implied that both promoter and genebody methylation could affect gene expression. We analyzed the effects of the promoter CpG islands (CGI) on gene expression and found that the expression level of genes without promoter CGI was significantly lower than that of genes with promoter CGI (Figure 4D). Meanwhile, we found genes with low methylation modification at promoter CGI had significantly higher expression levels than genes with high methylation modification at promoter CGI (Figure 4E), implying the methylation of CpGI also regulated the gene expression in MSCs.

Differential genes between BMMSCs and UCMSCs

We next compared the DNA methylation and gene expression differences between porcine BMMSCs and UCMSCs. 587 genes showing differentially methylated at the promoter region were identified, 280 genes were hypermethylated and 307 genes were hypomethylated in UCMSCs (Additional File 1: Table S1). Gene Ontology (GO) enrichment analysis revealed that the hypermethylated genes were significantly associated with skeletal system development, pattern specification process, and chordate embryonic development (Figure 5A). While the hypomethylated genes were significantly enriched in regulation of amine transport, regulation of catecholamine secretion, regulation of system process, and G-protein signaling, coupled to cyclic nucleotide second messenger (Figure 5B).

We also identified 1,979 differentially expressed genes (DEGs) between BMMSCs and UCMSCs (Additional File 1: Table S2). Compared with BMMSCs, 1,407 genes were up-regulated while 572 genes were down-regulated in UCMSCs. GO enrichment analysis revealed that the up-regulated genes were significant enriched in nuclear division, mitosis, organelle fission and cell cycle (Figure 5C), implying that UCMSCs have higher cell proliferative activity than BMMSCs. The down-regulated genes were significant enriched in skeletal system development, translational elongation cell migration, cell adhesion, ossification, and metabolic related processes (Figure 5D). These differential genes further revealed the characteristics of MSCs that depended on different cellular sources.

We found 102 genes that have both expression and promoter methylation differences. 36 of these genes were hypermethylated and down-regulated in BMMSCs, including C8ORF73, AOC3, FGF21, AC005841.1, CLDN4, TRPV2, MUC20, SERPINB5, CACNA1G, KCNH2, MCAM, BVES, ULBP3, CSMD2, PCDHGA7, TMEM200B, HTR1B, SLC22A18, CTF1, GPR44, CLSTN3, GPSM3, SPRY4, HOXD11, HOXC5, KIAA0895, CNTFR, ZBTB39, PEMT, FOXL1, FUT1, PMEPA1, RCSD1, DAB2IP, TNFRSF10B, AC024575.1. Meanwhile, 15 of them were hypermethylated and down-regulated in UCMSCs, including GATM, ADAMTS16, LPAR1, ITIH5, CFI, PTN, MLANA, FCRL1, CWH43, PAM, MOXD1, C6orf204, ARNTL2, SYN1, SLC9A9.

Validation of the MeDIP-seq and RNA-seq data

The methylation degree of 31 differentially methylated regions in the promoter of 15 genes was verified by Sequenom MassArray methylation analysis (Figure 6 and Additional File 1: Table S3), the expression level of 12 DEGs was validated by real-time quantitative PCR (RT-qPCR, Figure 6 and Additional File 2: Figure S1). These results were in accordance with the MeDIP-seq and RNA-seq results, confirming the reliability of our omic data.

Discussion

The biological characteristics of mesenchymal stem cells (MSCs) derived from different sources are different in proliferation, differentiation and migration abilities that affect their tissue repair capacity (Kolf et al., 2007; Shi et al., 2010; Li and Hua, 2017). Porcine MSCs can be easily obtained and share similar morphology and differentiation potential with human MSCs. The inbred WZSP line is an ideal large animal model with high genetic stability (Fang et al., 2012), which provides excellent materials for understanding the molecular characteristics of MSCs. To explore the biological characteristics and regulation mechanism of MSCs derived from different sources, we isolated BMMSCs and UCMSCs from WZSP, and profiled the genome-wide DNA methylome and transcriptome maps of these two MSCs.

Our results indicated that the porcine MSCs had similar DNA methylation patterns with other pig tissues (Li et al., 2012; Yang et al., 2016; Yang et al., 2017): TSS maintained a low methylation status and gene body exhibited a much higher level of DNA methylation than the 5' and 3' flanking regions. Genome-wide integrated maps of DNA methylation and transcriptome of porcine MSCs showed that the expression of genes was affected by both promoter and genebody methylation, confirming that promoter methylation repress gene expression (Jones, 2012; Smith et al., 2014). Most CpGs in the mammalian genomes are methylated, whereas CpGs within CGIs are usually unmethylated. While methylated CGIs are also observed during normal biological processes, such as X chromosome inactivation and gene imprinting (Cottrell, 2004). In this study, we found that the expression level of gene without promoter CGI was significantly lower than that of gene with promoter CGI. Additionally, methylation level of the promoter CGI had a negative correlation with gene expression level. These results indicated the methylation of CpGI might regulate the gene expression in MSCs, but their regulation mechanism still need to be further explored.

MSCs derived from different sources also manifested unique molecular characteristics. We identified 587 genes displaying promoter methylation differences and 1,979 genes displaying expression differences were compared between BMMSCs and UCMSCs. 102 genes had both expression and promoter methylation differences. Enrichment analysis revealed these differential genes were functionally related to the biological characteristics of MSCs. Skeletal system development was the most significantly enriched biological process for both the hypermethylated genes (such as Homeobox genes) and the down-regulated genes (such as PTN, RBP4) in UCMSCs. Homeobox genes are master developmental

control genes that act at the top of genetic hierarchies regulating aspects of morphogenesis and cell differentiation in animals (Mark et al., 1997). Pleiotrophin (PTN) gene has a higher expression level and lower promoter methylation degree in BMMSCs, this gene plays an important role in bone formation by mediating the recruitment and attachment of osteoblasts/osteoblast precursors to the appropriate substrates for the deposition of new bone (Erlandsen et al., 2012). These results indicated that BMMSCs have much higher osteogenic differentiation potential than UCMSCs. Previous study also showed that osteoblast differentiation of UCMSCs was less efficient, even after addition of 1.25-dihydroxyvitamin D3, a potent osteoinductive substance (Majore, 2011).

Compared with UCMSCs, Inter-alpha (globulin) inhibitor H5 (ITIH5) gene had a higher expression level and lower promoter methylation degree in BMMSCs. *ITIH5* was highly expressed in human adipocytes and adipose tissue, its expression was higher in obese subjects and reduced during diet-induced weight loss (Anveden et al., 2012). Fibroblast growth factor 21 (FGF21), an endocrine regulator in lipid metabolism, caused a dramatic decline in fasting plasma glucose, fructosamine, triglycerides, insulin, and glucagon when administered daily for 6 weeks to diabetic rhesus monkeys (Murata et al., 2011) (Alexei et al., 2007). Compared with BMMSCs, ITIH5 and FGF21 had a higher expression level and lower promoter methylation degree in UCMSCs. The results illustrated that the adipogenic differentiation ability of BMMSCs was stronger than UCMSCs.

Meanwhile, we observed that cell cycle genes were significantly up-regulated and hypomethylated in UCMSCs, such as CTF1, DAB2IP and CACNA1G. Cardiostrophin 1 (CTF1) can stimulate the proliferation of cardiomyocytes (Stejskal and Ruzicka, 2008) and plays an important role in cardiac repair in the infarcted heart (Freed et al., 2005). DAB2 interacting protein (DAB2IP) is a newly described member of the Ras GTPase-activating protein family and plays an important role in maintaining cell homeostasis and regulating cell proliferation, survival, and death (Daxing et al., 2009). Calcium channel, voltage-dependent, T type, alpha 1G subunit (CACNA1G) is a T-type calcium channel gene, and the hypermethylation of CACNA1G has been shown in various human tumors, which potentially affects cell proliferation and apoptosis (Toyota et al., 1999). These results implied that UCMSCs have higher cell proliferative activity than BMMSCs.

The extent of tight junction formation is one of many factors that regulate motility, invasion, and metastasis. As a member of Claudins, claudin 4 (CLDN4) is required for the formation and maintenance of tight junction (Lin et al., 2013). The forkhead box L1 (FOXL1) protein belongs to the forkhead box (Fox) family of transcription factors. Over-expression of FOXL1 inhibits tumor cell growth, migration and invasion of renal and pancreatic cancer cells (Geng et al., 2013; Feng-Qiang et al., 2014). Compared with BMMSCs, both CLDN4 and FOXL1 displayed a higher expression level and lower promoter methylation degree in UCMSCs, demonstrating that these differential genes regulated the migration ability of porcine MSCs.

Additionally, G protein-coupled receptor 44 (GPR44) plays a major role in the activation and chemotaxis of Th2 cells, eosinophils, and basophils (Ishii et al., 2012). G-protein signaling modulator-3 (GPSM3) is

known to bind to Gαi·GDP subunits and free Gβ subunits during their biosynthetic path toward Gγ dimer formation. GPSM3 is an important regulator of monocyte function involved in the regulation of differentiation, chemotaxis, and survival in *vitro* and in *vivo*, its deficiency is protective in acute inflammatory arthritis (Giguere et al., 2013). UL16 binding protein 3 (ULBP3), as a MHC class I-related molecule, can bind to human cytomegalovirus glycoprotein UL16 and activate natural killer cells (Kubin et al., 2001). The lower expression and higher methylation of GPR44, GPSM3 and ULBP3 in UCMSCs compared with BMMSCs suggested that the two MSCs have different immunogenicity properties.

Abbreviations

BM: Bone marrow; UC: umbilical cord; MSCs: Mesenchymal stem cells; DAVID: Database for Annotation, Visualization and Integrated Discovery; WZSP: Wuzhishan pig; CpGi: Cytosine phosphate guanine island; FDR: False discovery rate; GO: Gene Ontology; RT-qPCR: Real-time quantitative PCR; RPKM: reads per kilobase per million reads; TSS: Transcription start site. TTS: transcription termination site.

Declarations

Ethics approval and consent to participate

All animal procedures were approved by the Animal Care and Use Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KL and YM conceived and designed the research. YY, ZL, WZ, LH contributed for data analysis. LH and TW performed molecular experiments. YM collected the samples and provided the necessary materials. YY, TW and KL contributed writing the manuscript. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Isolation and identification of porcine BMMSCs and UCMSCs. (A) the fibroblast-like morphology of porcine MSCs. (B) Flow cytometry analysis of the surface markers expression on MSCs. The fluorescence in the range of M1 was considered as positive cells which were recognized by directed antibody. Autofluorescence intensity was less than 101 and cells detected in this range were negative. (C) Osteogenic and adipogenic differentiation potential of porcine BMMSCs and UCMSCs. The calcium deposits in osteocytes and lipid droplets generated appeared in adipocytes were stained red with Alizarin Red and Oil Red O. Scale bars, 100 µm.

Figure 2

Landscape of the DNA methylome and transcriptome of porcine MSCs. The distribution of DNA methylation and gene expression levels throughout the pig chromosomes was profiled. To compare DNA methylation and transcriptional level of BMMSCs and UCMSCs, read depth was normalized to the overall average amount of reads in each sample. A 1 Mb sliding window was used to smooth the distribution. Repeat elements, CGI length, gene density, number of CpG and CpGo/e ratio were all calculated in a 1 Mb sliding window.

Figure 3

DNA methylation distribution around the genebody and flanking regions of porcine MSCs. The upstream and downstream 2 kb regions were split into 20 non-overlapping windows, respectively, and the genebody of each gene was split into 40 equal windows. The average alignment depth was calculated for each window. The Y-axis is the average reads depth for each window. TSS: Transcription start site. TTS: transcription termination site.

Figure 4

Promoter methylation and transcriptional repression in porcine MSCs. (A) The number of genes with promoter and (or) genebody methylation modifications in BMMSCs. (B) The number of genes showing promoter and (or) genebody methylation in BMMSCs. (C) Gene expression comparison among genes showing promoter and (or) genebody methylation. (D) Gene expression comparison between genes with

promoter CGI and genes without promoter CGI. (E) Gene expression comparison between genes with different methylation level at promoter CGI.

Figure 5

GO functional enrichment analysis of the differential genes between BMMSCs and UCMSCs. (A-B) The top 10 biological process terms significantly enriched for the hypermethylated (A) and hypomethylated (B) genes in UCMSCs compared with BMMSCs. (C-D) The top 10 biological process terms significantly enriched for the up-regulated (C) and down-regulated (D) genes in UCMSCs compared with BMMSCs.

Figure 6

Validation of RNA-seq and MeDIP-seq data by RT-qPCR and Sequenom massARRAY, respectively. The expression and promoter methylation levels of three representative genes (HOXB5, FGF21 and CYP26A1) were validated by RT-qPCR and Sequenom massARRAY, respectively. (A) HOXB5, (B) FGF21, (C) CYP26A1. The expression levels of these three genes in BMMSCs and UCMSCs are shown in the left. The right panels show the results of Sequenom massARRAY. Each dot corresponds to one CpG position in the genomic sequence. The colored bars summarize the methylation level at that position, blue indicates methylated (100%) and yellow indicates unmethylated (0%). The validation results of other differential genes or promoter regions are shown in Additional File 2: Figure S1 and Additional File 1: Table S3.

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