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Original Article

# Microarray analysis of long non-coding RNAs related to osteogenic differentiation of human dental pulp stem cells

Xinyu Hao [a,e,f], Dongfang Li [d,e,f], Dongjiao Zhang [b,e,f], Linglu Jia [c,e,f*]

[a] Department of Pediatric Dentistry, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University, Jinan, China
[b] Department of Implantology, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University, Jinan, China
[c] Department of Prosthodontics, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University, Jinan, China
[d] School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University, Jinan, China
[e] Shandong Key Laboratory of Oral Tissue Regeneration, Jinan, China
[f] Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Jinan, China

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**KEYWORDS**
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Stem cells

**Abstract** *Background/purpose:* Dental pulp stem cells (DPSCs) are candidate seed cells for bone tissue engineering, but the molecular regulation of osteogenic differentiation in DPSCs is not fully understood. Long non-coding RNAs (lncRNAs) are important regulators of gene expression, and whether they play roles in osteogenic differentiation of DPSCs requires more study.

*Materials and methods:* DPSCs were isolated and cultured. The mRNA and lncRNA expression profiles were compared through microarray assay between osteo-differentiated DPSCs and non-differentiated DPSCs. Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, Gene ontology (GO) analyses, and the mRNA-lncRNA co-expression analyses were performed for functional annotation of differentially expressed RNAs. Small interfering RNA (siRNA) was used to interfere the expression of lncRNA ENST00000533992 (also named smooth muscle-induced lncRNA or SMILR), a candidate regulator, then the osteogenic differentiation potential of DPSCs was analyzed.

*Results:* DPSCs were isolated and cultured successfully. The expression of 273 mRNAs and 184 lncRNAs changed significantly in DPSCs after osteogenic induction. KEGG analyses and GO analyses showed that the differentially expressed RNAs were enriched in several pathways and

* Corresponding author. School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University, No. 44-1, Wenhua Xi Road, Jinan, Shandong, 250012, People's Republic of China. Fax: +86 531 88382923.
*E-mail address:* danlingjia@163.com (L. Jia).

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biological processes. The mRNA-lncRNA co-expression network was constructed to reveal the potential relationships between mRNAs and lncRNAs. The osteogenic differentiation potential of DPSCs decreased when SMILR was interfered.
*Conclusion:* The present study provides clues for seeking for lncRNAs that participate in the regulation of osteogenic differentiation in DPSCs. LncRNA SMILR could play a role in regulating osteogenic differentiation of DPSCs.


## Introduction

Various of factors such as congenital malformations, tumors, periodontitis and trauma may lead to the defect of bone tissue in the oral and maxillofacial region. Since speaking, chewing, appearance and so on are closely related to the health of oral and maxillofacial bone tissues, promoting the regeneration of lost bone is a concern of many scholars and doctors.[1,2]

In recent years, bone tissue engineering technology using seed cells, growth factors and scaffold materials to regenerate bone tissue has made continuous progress.[3,4] Stem cells own self-renewal and multi-directional differentiation abilities, so they are considered as candidate seed cells for bone tissue engineering.[5,6] Dental pulp stem cells (DPSCs), a type of stem cells that were first isolated and identified from dental pulp tissues in 2000,[7] have been proved to have great bone regeneration potential in several animal and human studies.[8–10] On the one hand, "wisdom teeth" as medical waste can be the source of DPSCs, so the isolation and culture of DPSCs is much easier. On the other hand, DPSCs show considerable proliferation and osteogenic differentiation capabilities *in vitro*, which is conducive to the implementation of bone tissue engineering. However, the molecular mechanisms involved in the osteogenic differentiation process of DPSCs are complex and not fully understood. To promote DPSCs-based bone regeneration, it is necessary to further study the molecular regulation of osteogenic differentiation in DPSCs.

In the past, scientists paid more attention to the protein-coding parts of DNA and RNA. Recently, quantities of RNAs that do not encode proteins have been proved to play roles in regulating cell biological behaviors in a variety of physiological and pathological processes.[11] Long non-coding RNAs (lncRNAs) are a typical type of RNA molecules with a length of more than 200 nucleotides and do not encode proteins.[11–13] More and more lncRNAs have been found to interact with DNA, RNA or protein molecules to regulate the expression of coding genes, and then affect the process of osteogenic differentiation of stem cells.[14–17] In DPSCs, although some lncRNAs such as ANCR,[18] H19,[19] THAP9-AS1,[20] CCAT1,[21] and MALAT1[22] have been proved to be involved in the regulation of osteogenic differentiation, whether other lncRNAs have regulatory effects remains to be screened and studied.

The microarray technology has provided efficient and convenient tools to analyze nucleic acid expression profiles of samples.[23–25] In the present study, microarray assay was applied to analyze the expression of lncRNAs and mRNAs in DPSCs after osteogenic differentiation, and those with large change in expression level were further analyzed by bioinformatic methods. Finally, the function of candidate regulator lncRNA ENST00000533992 (also named smooth muscle-induced lncRNA or SMILR) was preliminarily explored. We hope our results could offer clues for molecular mechanism studies in DPSCs.

## Materials and methods

### DPSCs isolation and culture

The ethics committee of Shandong university approved all operations y (approval number: GR201603). Healthy premolars or the third molars that were extracted for orthodontic treatment were collected from 16 to 25 years old patients in Stomatology Hospital of Shandong University. Informed consent was obtained from patients. The teeth were cleaned and sectioned carefully, and then the dental pulp tissues located in pulp cavity were gained. After the tissue near the root tip was removed, the dental pulp was cut into small pieces, and then were digested in 3 mg/mL collagenase I (Sigma—Aldrich, St. Louis, MO, USA) and 4 mg/mL dispase (Sigma—Aldrich) at 37 °C for 1 h. After filtration and centrifugation, the cells were seeded into culture dishes with cell culture medium, which was α-MEM (Corning Incorporated, Corning, NY, USA) supplemented with 10% fetal bovine serum (Corning). The cells were cultured in a 5% $CO_2$ incubator at 37 °C, and cells were passaged when reaching the confluence of 80%.

### Osteogenic differentiation detection

DPSCs were cultured in osteogenic induction medium, which contained α-MEM, 10% fetal bovine serum, 10 nmol/L dexamethasone (Solarbio, Beijing, China), 10 mmol/L β-glycerophosphate (Solarbio) and 50 mg/L ascorbic acid (Solarbio). The medium was refreshed every 3 days. After 21 days, DPSCs were fixed with 4% paraformaldehyde, then were stained with Alizarin Red to detect the formation of mineralized nodules.

### Adipogenic differentiation detection

DPSCs were cultured in adipogenic induction medium, which contained α-MEM, 10% fetal bovine serum, 0.5 mmol/

L isobutyl-methylxanthine (Solarbio), 0.2 mmol/L indomethacin (Solarbio), 1 μmol/L dexamethasone (Solarbio) and 0.01 g/L insulin (Solarbio). The medium was refreshed every 3 days. After 28 days, DPSCs were fixed with 4% paraformaldehyde, then were stained with Oil Red O to detect the formation of lipids.

### Chondrogenic differentiation detection

DPSCs were cultured in chondrogenic induction medium (Cyagen, Santa Clara, CA, USA) in the form of cell mass. The medium was refreshed every 3 days. After 28 days, DPSCs mass were prepared into paraffin sections and were stained with Alcian Blue to detect the formation of cartilage-like matrix.

### Immunophenotype identification

The imunophenotype of DPSCs was detected by flow cytometry. All operations were carried out according to the instructions of the BD Human MSC Analysis Kit (BD Biosciences, Franklin Lakes, NJ, USA), and the expression of CD90, CD44, CD105, CD73, CD34, CD11b, CD19, CD45 and HLA-DR was analyzed.

### DPSCs samples preparation and microarray assay

Three DPSCs clones isolated from three different individuals were prepared for microarray analyses. In the experimental group, all DPSCs clones were cultured in osteogenic induction medium for 7 days, then the total RNA of cells was isolated by RNAios Plus reagent (Takara, Tokyo, Japan). In the control group, the total RNA was collected after DPSCs clones were cultured in cell culture medium for 7 days. The GeneChip Human Transcriptome Array 2.0 (Affymetrix, Santa Clara, CA, USA) and GCBI platform (GMINIX Informatics Ltd. Co, Shanghai, China) were used to analyze all RNA samples, then the differentially expressed mRNAs and lncRNAs between the experimental group and the control group were screened according to requirements of fold change (FC)$\geq$1.5 and $p < 0.05$.

### Bioinformatics analyses

The potential function of genes and their products were analyzed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and the Gene Ontology (GO) database. In KEGG analyses, the enrichment degree of genes in different pathway terms were calculated and compared. In GO analyses, three fields including cellular component (CC), biological process (BP) and molecular function (MF) were included.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA was isolated from cells by RNAios Plus reagent (Takara) according to the instruction of manufacture. After the purity and concentration of RNA were measured by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), RNA was reverse transcribed to cDNA by PrimeScript ™ RT reagent Kit (Takara). The qRT-PCR was performed with TB green (Takara) by Roche LightCycler®480II. GAPDH was used as the internal control. The sequences of primers are listed in supplemental file 1.

### Construction of mRNA-lncRNA co-expression network

To analyze the potential correlation between mRNAs and lncRNAs that have significantly change in expression level, Hybrid hierarchical clustering algorithm was used to calculate the correlation coefficient of mRNA-lncRNA pair, then the mRNA-lncRNA co-expression network was constructed.

### Small interfering RNA (siRNA) transfection

DPSCs were transfected with siRNA (Sense 5′-CCUAAACUCGAAUCUGAUUTT - 3′, Antisense 5′-AAUCAGAUUCGAGUUUAGGTT - 3′) to reduce the expression of lncRNA SMILR using the Micropoly-transfecter Cell Reagent (Micropoly, Xian, Shanxi, China). DPSCs transfected with non-target siRNA (Sense 5′- UUCUCCGAACGUGUCACGUTT- 3′, Antisense 5′- ACUUGACACGUUCGGAGAATT- 3′) were used as control group. All DPSCs were cultured in the osteogenic medium for 7 days (DPSCs were transfected again on the fourth day to enhance the effect of siRNA), then were collected for Alkaline phosphatase (ALP) staining (by NBT/BCIP staining kit (Beyotime, Shanghai, China)), ALP activity assay (by ALP assay kit (Jiancheng, Nanjing, China)) and qRT-PCR experiments.

### Statistical analyses

All experiments were repeated at least three times. Fisher exact test, Pearson correlation, and two sample t test were used to determine statistical differences. $p < 0.05$ was considered statistically significant.

## Results

### Culture and multi-differentiation ability detection of DPSCs

The primary DPSCs cultured *in vitro* were fibroblast-like morphology and had clonogenic ability (Fig. 1A). After being passaged, DPSCs maintained a fibroblast-like appearance and showed good proliferation capability (Fig. 1B). The results of Alizarin Red staining proved that DPSCs could form mineralized nodules after osteogenic induction (Fig. 1C). Lipids were observed by Oil Red O staining in DPSCs after adipogenic induction (Fig. 1D). The results of Alcian Blue staining indicated that DPSCs generated cartilage-like matrix after chondrogenic induction (Fig. 1E). The above detections supported that DPSCs owned the ability of multidirectional differentiation.

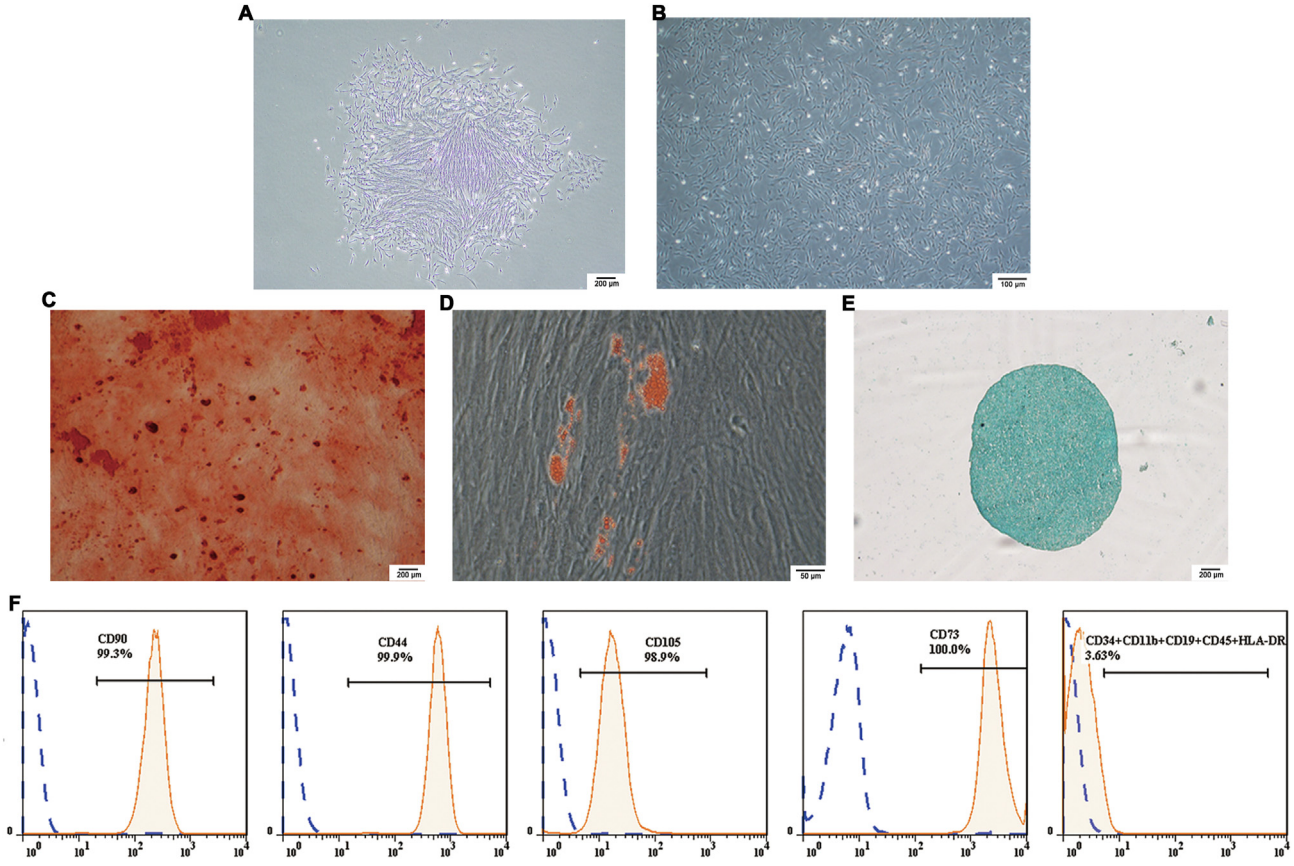


**Figure 1 Culture and identification of DPSCs.** (A) Primary cultured DPSCs. (B) DPSCs at passage 3. (C) Alizarin Red staining of DPSCs that cultured in osteogenic induction medium for 21 days. (D) Oil Red O staining of DPSCs that cultured in adipogenic induction medium for 28 days. (E) Alcian Blue staining of DPSCs that cultured in chondrogenic induction medium for 28 days. (F) Immunophenotype identification of DPSCs. The expression of CD90, CD44, CD105, CD73, CD34, CD11b, CD19, CD45 and HLA-DR was analyzed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

## Immunophenotype identification of DPSCs

The results of flow cytometry proved that DPSCs were positive to mesenchymal stem cell surface markers (CD90, CD44, CD105, CD73), and were negative to hematopoietic and endothelial cell surface markers (CD34, CD11b, CD19, CD45, HLA-DR) (Fig. 1F). These results showed that DPSCs accorded with the characteristics of mesenchymal stem cells.

## Screening of differentially expressed mRNAs and lncRNAs

The RNA expression profiles were compared through microarray assay between osteo-differentiated DPSCs and non-differentiated DPSCs. In total, the expression level of 273 mRNAs changed significantly after osteogenic induction, including 162 upregulated ones and 111 downregulated ones (Fig. 2A and B, supplemental file 2). At the same time, 184 lncRNAs were differentially expressed during osteogenic induction, of which 97 were upregulated and 87 were downregulated (Fig. 2C and D, supplemental file 3). The mRNAs and lncRNAs with most significant changes in expression levels were shown in Table 1 and Table 2. To verify the results of microarray assay, qRT-PCR was used to detect the expression of 6 lncRNAs and 6 mRNAs in osteo-differentiated and non-differentiated DPSCs. As shown in Fig. 3, the results of qRT-PCR and microarray analyses were consistent.

## KEGG analyses and GO analyses

In KEGG analyses, the differentially expressed mRNAs between osteo-differentiated DPSCs and non-differentiated DPSCs were mainly enriched in 68 signaling pathway terms, such as alcoholism, mineral absorption, metabolic pathways, and aminoacyl-tRNA biosynthesis. According to *p* value, the top 20 pathways were shown in Fig. 4A. In GO analyses, mRNAs with significant changes in expression level were significantly enriched in 94 BP terms, 46 MF terms and 40 CC terms. The most enriched BP terms contained cellular response to zinc ion, negative regulation of growth, small molecule metabolic process, extracellular matrix organization and so on (Fig. 4D). MF terms such as protein binding, ATP binding, and calcium ion binding had more gene enrichment (Fig. 4C). As for CC, cytoplasm,

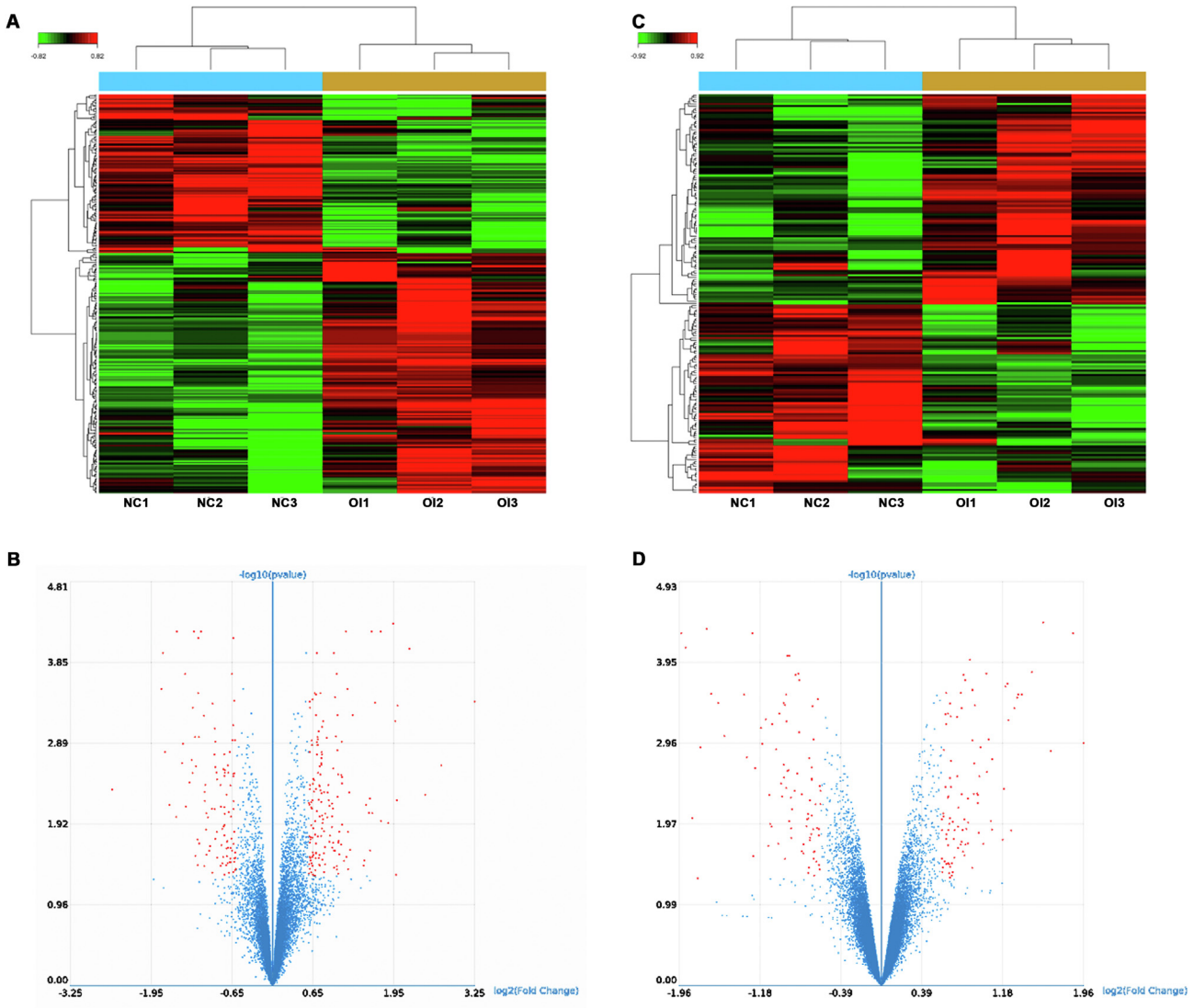


**Figure 2 Microarray assay of mRNA and lncRNA expression profiles of DPSCs.** (A) Clustering heatmap of differentially expressed mRNAs in DPSCs. (B) Volcano Plot of differentially expressed mRNAs in DPSCs. According to the requirements of $p < 0.05$ and fold change$\geq$1.5 , 273 mRNA were differentially expressed between osteo-differentiated and non-differentiated DPSCs. (C) Clustering heatmap of differentially expressed lncRNAs in DPSCs. (D) Volcano Plot of differentially expressed lncRNAs in DPSCs. According to the requirements of $p < 0.05$ and fold change$\geq$1.5 , 184 lncRNAs were differentially expressed between osteo-differentiated and non-differentiated DPSCs. NC: the negative control DPSCs that were cultured in cell culture medium for 7 days. OI: the experimental DPSCs that were cultured in osteogenic induction medium for 7 days.

extracellular matrix, nucleus and so on were the top terms (Fig. 4B). These results provided clues for further study on the regulation mechanisms of osteo-differentiation in DPSCs.

## Construction of mRNA-lncRNA co-expression network

Based on the results of correlation assay, the mRNA-lncRNA co-expression network was constructed (Fig. 5). It can be seen that hundreds of differentially expressed mRNAs and lncRNAs after osteogenic induction in DPSCs exhibited potential positive or negative relationships. The mRNAs or lncRNAs with a large connectivity number may play a role in the gene regulatory network of DPSCs during osteogenic differentiation, which requires further experiments to verify.

## SMILR interference inhibited the osteogenic differentiation of DPSCs

Based on the fold change, nucleotide sequence, molecular weight, and literature reports of all lncRNAs, we screened SMILR, whose expression level increased after osteogenic differentiation in DPSCs, as the following experiment target. After using siRNA to reduce the expression level of SMILR terms (Fig. 6A), we evaluated the changes of osteogenic differentiation ability of DPSCs. The results of qRT-

**Table 1** Top 10 differentially expressed mRNAs between osteo-differentiated DPSCs and non-differentiated DPSCs.

| Expression level | Gene Symbol | Accession Number | Database Source | Fold Change | *p*-value |
|---|---|---|---|---|---|
| up | PIP | NM_002652 | RefSeq | 9.49968 | 0.00042 |
| | FKBP5 | NM_004117 | RefSeq | 6.55553 | 0.00240 |
| | DIO2 | NM_000793 | RefSeq | 5.47643 | 0.00535 |
| | TIMP4 | NM_003256 | RefSeq | 4.59626 | 0.00010 |
| | MAOA | NM_000240 | RefSeq | 4.02474 | 0.00047 |
| down | ASNS | NM_001178075 | RefSeq | −3.15856 | 0.00706 |
| | TGFBI | NM_000358 | RefSeq | −3.31833 | 0.00165 |
| | ITGA11 | NM_001004439 | RefSeq | −3.39276 | 0.00011 |
| | VLDLR | NM_001018056 | RefSeq | −3.45250 | 0.00029 |
| | PSAT1 | NM_058179 | RefSeq | −5.96615 | 0.00465 |

DPSCs: dental pulp stem cells; mRNA: messenger RNA; PIP: prolactin induced protein; FKBP5: FK506-binding protein 5; DIO2: iodothyronine deiodinase 2; TIMP4: metallopeptidase inhibitor 4; MAOA: monoamine oxidase A; ASNS: asparagine synthetase; TGFBI: transforming growth factor beta induced; ITGA11: integrin subunit alpha 11; VLDLR: very low density lipoprotein receptor; PSAT1: phosphoserine aminotransferase 1.

**Table 2** Top 10 differentially expressed lncRNAs between osteo-differentiated DPSCs and non-differentiated DPSCs.

| Expression level | Gene Symbol | Accession Number | Database Source | Fold Change | *p*-value |
|---|---|---|---|---|---|
| up | — | n339249 | NONCODE | 3.90229 | 0.00109 |
| | — | TCONS_00016019-XLOC_007414 | Rinn lincRNA | 3.64351 | 0.00005 |
| | — | n341454 | NONCODE | 3.13560 | 0.00138 |
| | — | n341703 | NONCODE | 2.97363 | 0.00004 |
| | — | NR_001447 | RefSeq | 2.76071 | 0.00015 |
| down | — | n342266 | NONCODE | −3.38075 | 0.00124 |
| | — | TCONS_00014189-XLOC_006198 | Rinn lincRNA | −3.44431 | 0.04919 |
| | — | n339452 | NONCODE | −3.57225 | 0.00903 |
| | — | n336648 | NONCODE | −3.74063 | 0.00008 |
| | — | n337911 | NONCODE | −3.85470 | 0.00005 |

DPSCs: dental pulp stem cells; lncRNA: long non-coding RNA.

PCR showed that the expression level of RUNX2 decreased slightly when SMILR was interfered (Fig. 6B). The results of ALP activity assay (Fig. 6C) and ALP staining (Fig. 6D and E) showed that the activity of ALP in siSMILR DPSCs was weaker than that of control cells. Thus it could be seen that SMILR interference inhibited the osteogenic differentiation of DPSCs, suggesting that SMILR might participate in the regelation of osteogenic differentiation in DPSCs.

## Discussion

DPSCs have important application value in bone tissue engineering, so understanding the molecular regulation mechanism of them is conducive to their application.[8] In addition to the genes that can encode protein, more and more non-coding genes have been proved to play regulatory roles in the osteogenic differentiation of stem cells.[26–28] Therefore, we compared the expression files of coding and non-coding genes between osteo-differentiated and non-differentiated DPSCs through microarray assay, and screened 273 mRNAs and 184 lncRNAs that changed significantly in expression level after osteogenic induction. Up to now, the potential function of these differentially expressed mRNAs have been revealed to some extent, and some of them have been proved to play roles in regulating cell osteogenic differentiation. For example, prolactin-inducible protein (PIP), the expression of which changed significantly in DPSCs after osteogenic induction, were reported to be related with cell behaviors of breast cancer and osteogenic differentiation of periodontal ligament stem cells.[29,30] Integrin subunit alpha 11(ITGA11), another mRNA with large change in expression level, was proved to affect the osteogenesis of bone marrow stem cells.[31,32] Some other mRNAs such as FK506-binding protein 5 (FKBP5), iodothyronine deiodinase 2 (DIO2), and metallopeptidase inhibitor 4 (TIMP4), were also differentially expressed in osteo-differentiated DPSCs. However, it is still unclear whether they can regulate the osteogenic differentiation of DPSCs, or their expression level changed only due to osteogenic differentiation. As for those differentially expressed lncRNAs, most of their functions have not been fully studied. Therefore, more experiments are needed to verify the roles of these mRNAs and lncRNAs.

Actually, some previous studies have used similar methods to seek for potential regulators in DPSCs: Liu cultured DPSCs in osteogenic/odontogenic medium for 14 days and found a total of 89 lncRNAs, and 1636 mRNAs were differentially expressed after differentiation.[33] Chen identified 132 differentially expressed lncRNAs and 172

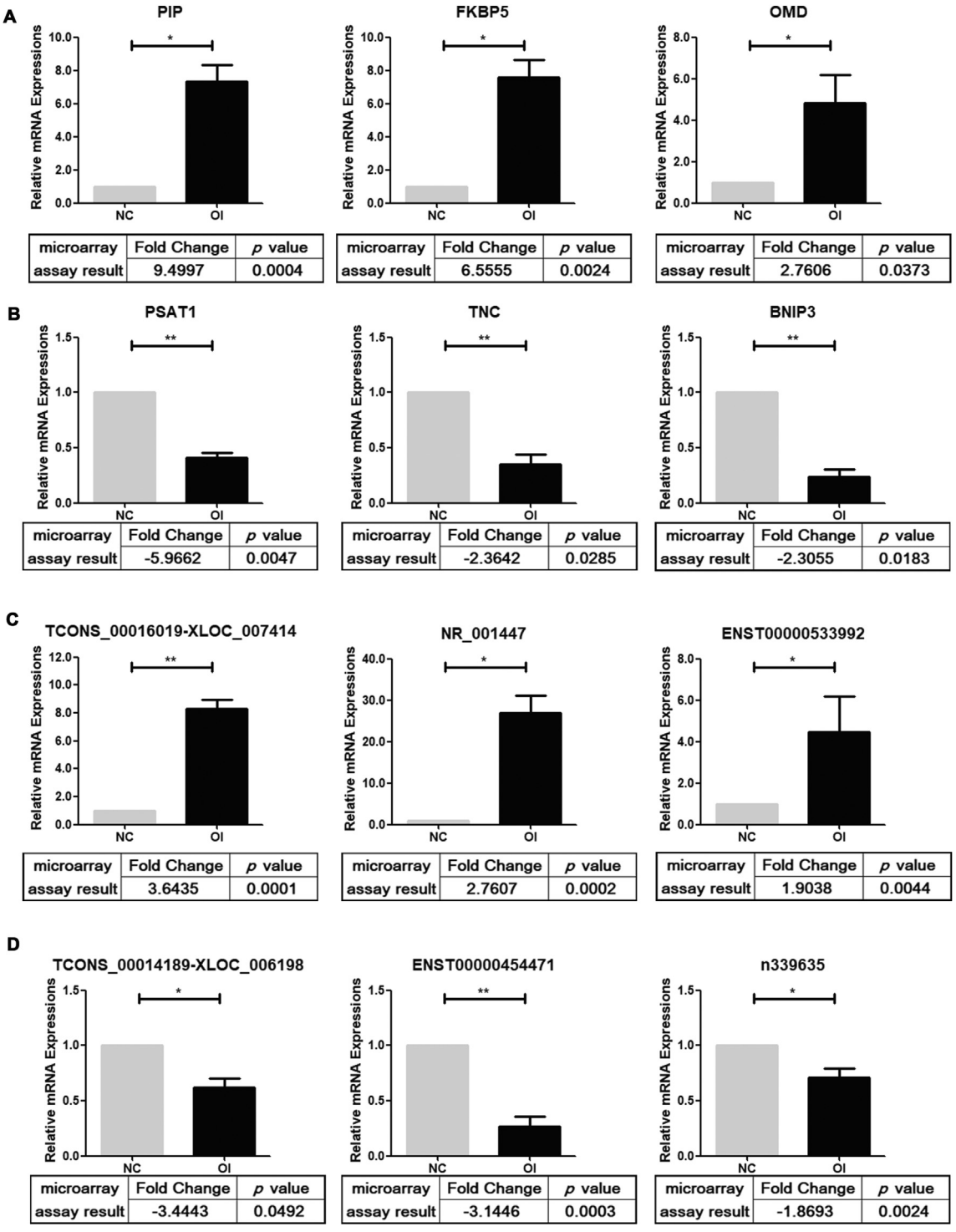


**Figure 3 Gene expression detected by qRT-PCR in DPSCs.** The qRT-PCR was used to validate the expression data of mRNAs and lncRNAs from microarray assay. (A) The expression of up-regulated mRNAs (PIP, OMD, FKBP5) was detected by qRT-PCR. (B) The expression of down-regulated mRNAs (PSAT1, TNC, BNIP3) was detected by qRT-PCR. (C) The expression of up-regulated lncRNAs (TCONS_00016019-XLOC_007414, NR_001447, ENST00000533992(SMILR)) was detected by qRT-PCR. (D) The expression of down-regulated lncRNAs (n339635, TCONS_00014189-XLOC_006198, ENST00000454471) was detected by qRT-PCR. PIP: prolactin induced protein; FKBP5: FK506-binding protein 5; OMD: osteomodulin; PSAT1: phosphoserine aminotransferase 1; TNC: tenascin C; BNIP3: BCL2 interacting protein 3. $^{*}P < 0.05$, $^{**}P < 0.01$.

differentially expressed mRNAs after DPSCs were cultured in odontogenic medium for 14 days.[34] Different from these previous studies, we paid more attention to the early stage of osteogenic differentiation, so we analyzed DPSCs that had been cultured in osteogenic induction medium for 7 days. The differences in cell samples, cell processing

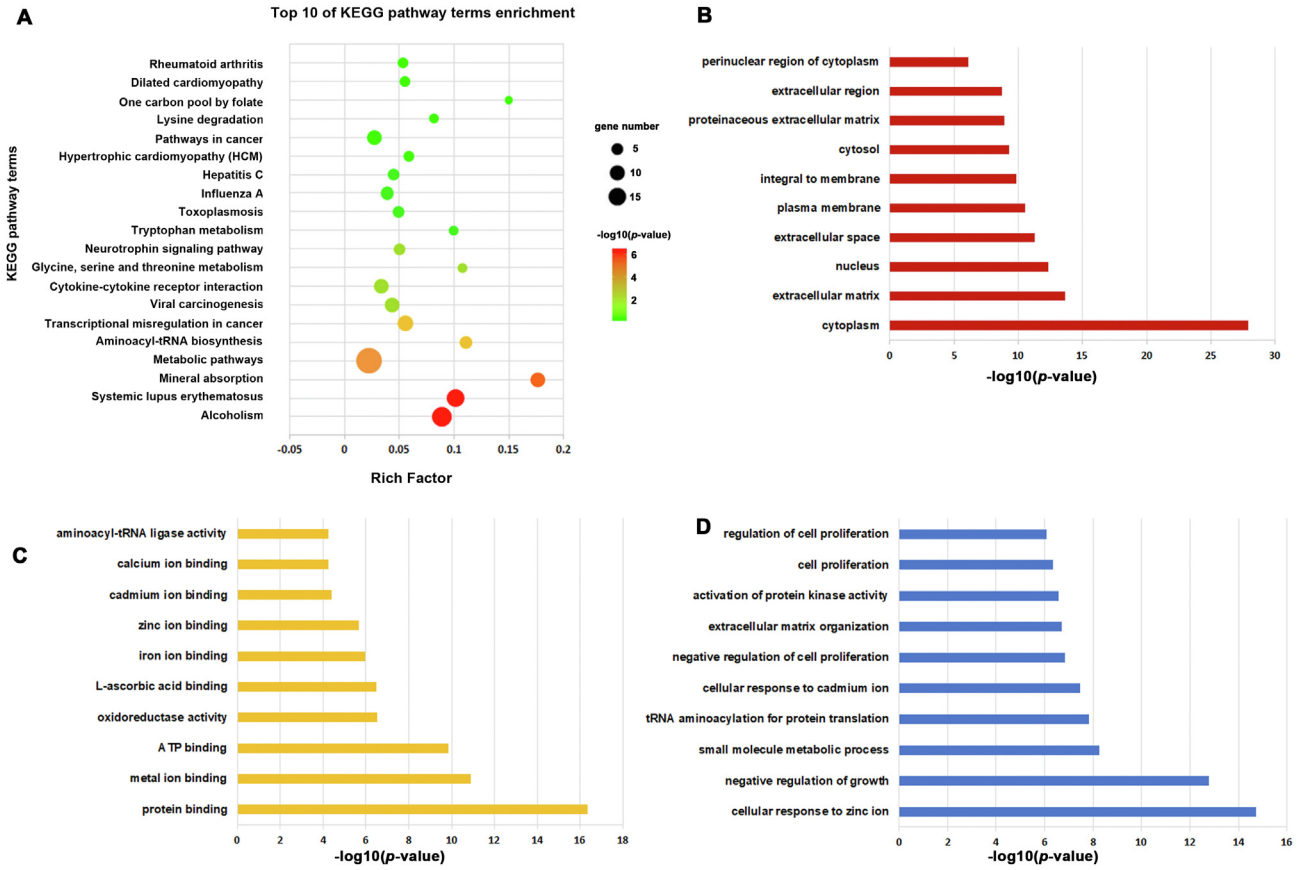


**Figure 4 KEGG and GO analyses of differentially expressed mRNAs in DPSCs.** (A) The top 20 KEGG pathways enriched by differentially expressed mRNAs in DPSCs. (B) The top 10 cellular component terms enriched by differentially expressed mRNAs in DPSCs. (C) The top 10 molecular function terms enriched by differentially expressed mRNAs in DPSCs. (D) The top 10 biological process terms enriched by differentially expressed mRNAs in DPSCs.

methods, sensitivity of detection technology may be the reasons for the inconsistent results of studies from different laboratories. It is best to analyze the results of different studies comprehensively and verify them by more experiments, so as to provide clues for clarifying the molecular mechanism of osteogenic differentiation in DPSCs.

According to the KEGG and GO database, the potential function of the differentially expressed mRNAs in DPSCs were analyzed. As mentioned above, these mRNAs were enriched in several metabolic and energy related biological processes or signaling pathways, which suggested that there may be a large number of energy regulation and small molecule metabolism changes in the early stage of osteogenic differentiation. These results were similar with some previous studies.[33,34] In addition, extracellular matrix organization biological processes and mineral absorption signaling pathways also had high enrichment score. We speculated that the remodeling and mineralization of extracellular matrix were of great significance for osteogenic differentiation.

The construction of mRNA-lncRNA co-expression network revealed the potential positive or negative relationships among differentially expressed mRNAs and lncRNAs in DPSCs. For instance, the network showed that several lncRNAs such as ENST00000528204, n342098, and n406510 were positively correlated with PIP. As mentioned earlier, PIP could be related to the osteogenic differentiation of DPSCs, so the connection between these lncRNAs and the process of osteogenic differentiation is worth exploring. In addition, the co-expression network built a bridge between lncRNAs and GO or KEGG analyses, which help preliminarily predict the function of some lncRNAs. We can see that the mRNAs that enriched in extracellular matrix organization biological process were correlated with several lncRNAs including TCONS_00006523-XLOC_003146, n384265, n340869 and so on; the mRNAs that enriched in metabolic pathways were correlated with lncRNAs such as n342369, ENST00000379816, and n342369. Anyway, although the co-expression network provided references for exploring the function of lncRNAs, more functional experiments are needed to verify the exact regulatory roles of them.

Among all differentially expressed lncRNAs, ENST00000533992, which was also called smooth muscle-induced lncRNA (SMILR), drew our attention. SMILR is a lncRNA that contains 553 nucleotides and does not encode proteins. It was firstly reported by Ballantyne in 2016, and was identified as a driver of vascular smooth muscle cell proliferation.[35] The following studies revealed that SMILR could regulate cell proliferation in many ways: SMILR was revealed to affect the proliferation of vascular smooth muscle cells by regulating late mitotic protein CENPF.[36]

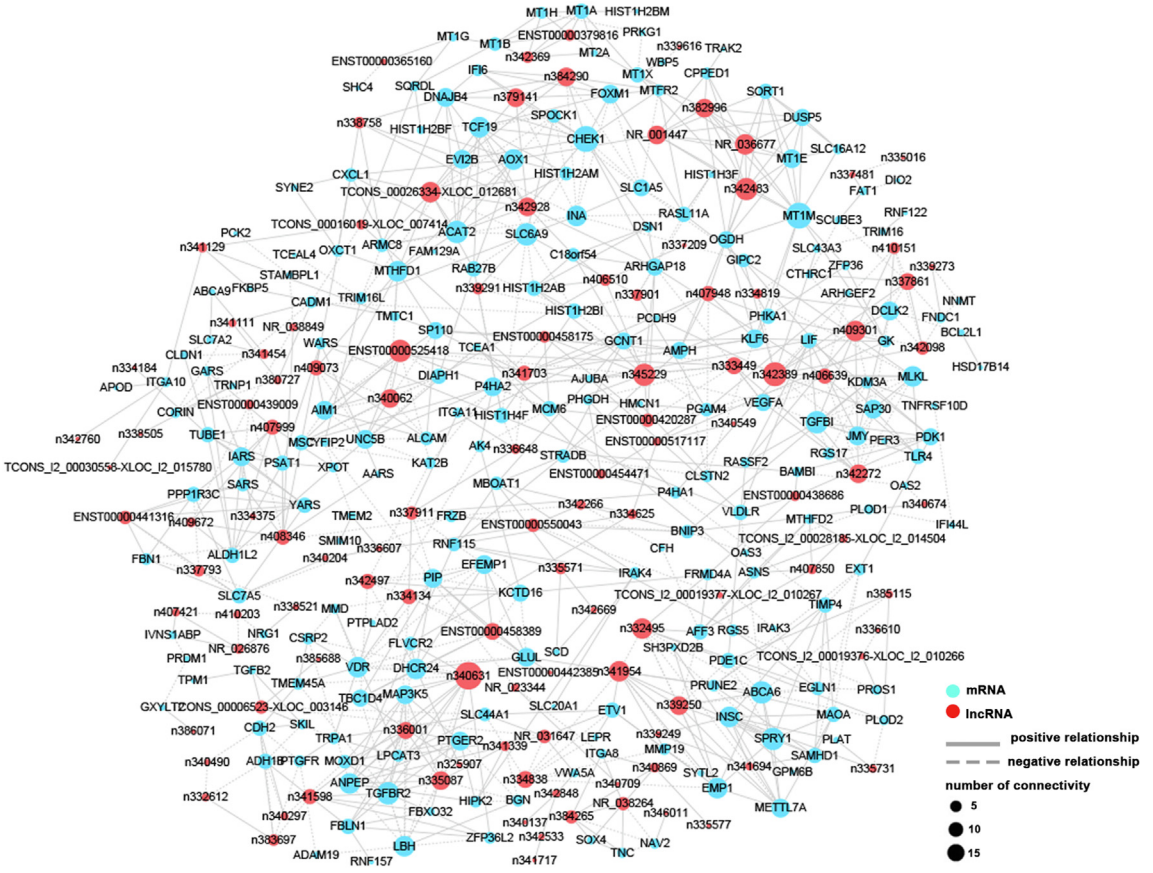


**Figure 5 The mRNA-lncRNA co-expression network in DPSCs.** The mRNA-lncRNA co-expression network in DPSCs was constructed. Blue nodes represent mRNAs. Red nodes represent lncRNAs. The solid lines represent positive correlations. The dashed lines represent negative correlations. The size of nodes represents number of connectivity, and larger nodes have more connection relationships.

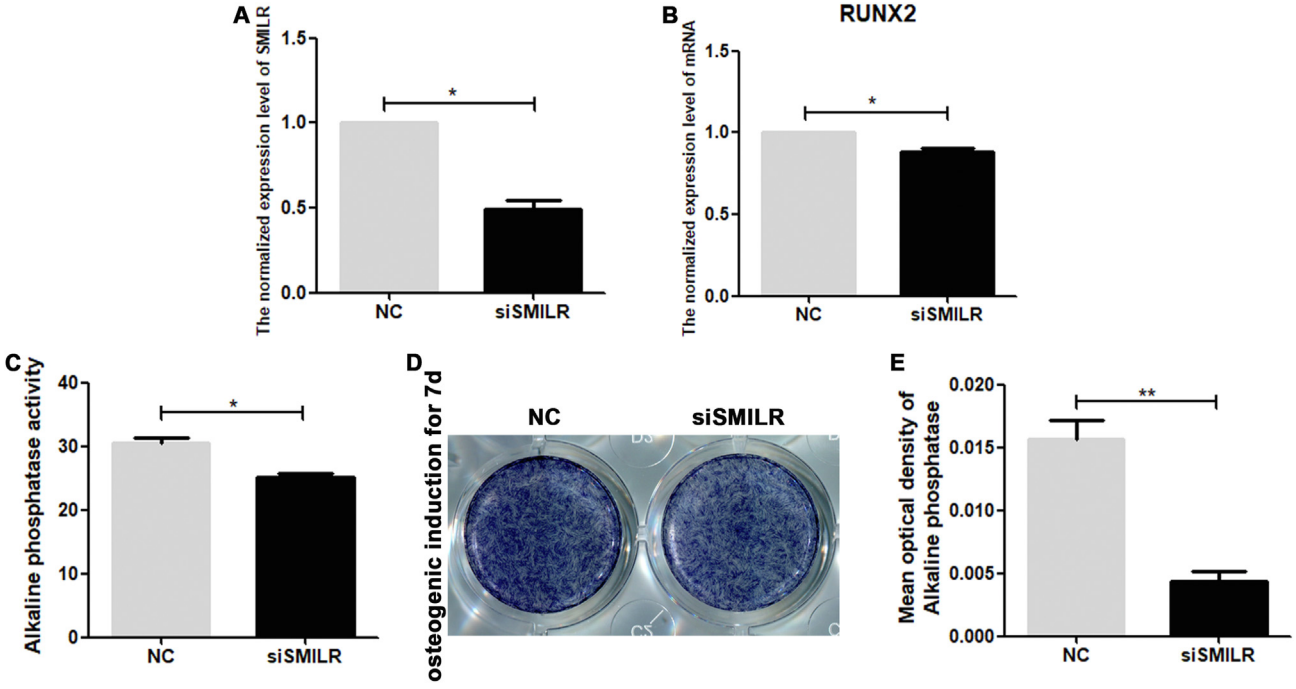


**Figure 6 SMILR interference inhibited the osteogenic differentiation of DPSCs.** (A) siRNA was used to interfere the expression of SMILR, and qRT-PCR was used to detect the expression of SMILR. (B) The expression of RUNX2 in DPSCs was detected by qRT-PCR after SMILR was interfered. (C) Alkaline phosphatase activity assay of DPSCs after SMILR was interfered. (D) Alkaline phosphatase staining of DPSCs after SMILR was interfered. (E) Quantitative analysis of alkaline phosphatase staining. $^{*}p < 0.05$.

Another report proved that SMILR participated in the progression of atherosclerosis by targeting the miR-10b-3p/KLF5 axis.[37] SMILR could also regulate the migration and proliferation of pulmonary arterial smooth muscle cells through miR-141/RhoA/ROCK pathway, thus affect the development of pulmonary arterial hypertension.[38,39] Recently, the expression level of SMILR was reported to be related to the survival rate of patients with gastrointestinal neuroendocrine tumors,[40] suggesting that SMILR may have other functions. Our present study showed that the expression of SMILR in DPSCs changed significantly after osteogenic induction. When using siRNA to interfere the expression of SMILR, the ALP activity of DPSCs decreased, and the expression of osteogenesis-related RUNX2 decreased slightly. These results indicated that SMILR could affect the osteogenic differentiation potential of DPSCs to some extent. However, the regulatory effect of SMILR on osteogenesis needs to be further verified, including adding more detection indicators, prolonging the time of osteogenic induction, and exploring the molecular mechanism of it, which will become our future research content.

In conclusion, in this study the mRNAs and lncRNAs with significant changes in expression level were analyzed by microarray and bioinformatics methods in DPSCs after osteogenesis induction. LncRNA SMILR could affect the osteogenic differentiation of DPSCs. All these results provided clues to seek for important lncRNAs that regulate the osteogenic differentiation process of DPSCs.

## Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2021.10.014.

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