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Profiling lncRNA alterations during TNF-α induced osteogenic differentiation of dental pulp stem cells

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Abstract. The multipotent and easily accessible characteristics of dental pulp stem cells (DPSCs) make them a promising target for bone tissue engineering. Long non-coding RNAs (lncRNAs) have an important role in the osteogenic differentiation of mesenchymal stem cells. Nevertheless, whether lncRNAs are involved in the osteogenic differentiation of DPSCs remains unclear. The present study examined the expression alterations of lncRNAs in tumor necrosis factor-α induced osteogenic differentiation of DPSCs. Following identification of differentially expressed lncRNAs at different time points by reverse transcription-quantitative polymerase chain reaction, profiling analysis was performed and a profile was further validated, in which lncRNA expression levels demonstrated significant upregulation. The next generation sequencing analysis identified 77 (58 upregulated and 19 downregulated) and 133 differentially expressed lncRNAs (73 upregulated and 60 downregulated) at 7 and 14 days post-treatment, respectively. In addition, 34 lncRNAs were predicted to be strongly associated with 336 mRNA transcripts that underwent significant alterations during osteogenic differentiation. The present data demonstrated that one lncRNA, X inactive specific transcript, is essential for efficient osteogenic differentiation of DPSCs by alkaline phosphatase staining. In summary, the present findings provide insight for the understanding of how non-coding RNAs are involved in regulating the osteogenic differentiation of DPSCs, which may further advance the translational studies of bone tissue engineering.

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Abbreviations: DPSCs, dental pulp stem cells; lncRNA, long non-coding RNA; DMEM, Dulbecco's modified Eagle's medium

Key words: DPSCs, RNA-sequencing, lncRNA, lncRNA X inactive specific transcript, tumor necrosis factor-α

Introduction

Dental pulp stem cells (DPSCs) are highly proliferative, multipotent, colorogenic type cells capable of multilineage differentiation and self-renewal which may be used for different regenerative medicine applications, including bone tissue engineering (1-3). DPSCs have a natural function in the production of odontoblasts and are capable of osteogenic differentiation (4-6). In previous studies, it was demonstrated that tumor necrosis factor-α (TNF-α) may successfully promote the transition between DPSCs and bone cells through step-wise, globally wide mRNA expressional alterations (7,8). Nevertheless, it remains unknown whether other types of mechanisms, including epigenetic regulation are involved in the osteogenic differentiation of DPSCs.

Accumulating evidence have identified that non-coding RNA transcripts, including microRNAs and long non-coding RNAs (lncRNAs) are essential in stem cell proliferation and differentiation (9-11). Different from mRNAs and microRNAs, lncRNAs are transcribed by RNA polymerase II; however, lack stable open reading frames (12-14). Functioning in the cis- or trans-manners, lncRNAs may either serve as a platform to recruit complex protein machinery to bind specific DNA loci or directly bind RNA molecules to implement post-transcriptional regulation (12-14). A number of lncRNAs have been demonstrated to serve regulatory roles in osteogenic differentiation from mesenchymal stem cells (15-17). Nevertheless, whether lncRNAs are involved in the osteogenic differentiation of DPSCs remains unclear.

The present study examined the involvement of lncRNAs in the osteogenic differentiation of DPSCs. By RNA-Sequencing (RNA-Seq) and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) validation, alterations in lncRNA expression at different phases of osteogenic differentiation were identified. Further analysis identified that one lncRNA, X inactive specific transcript (XISP), is required for this process. These findings provide insight for the understanding of *in vitro* induced differentiation of DPSC mechanisms, thus identifying potential molecular targets which promote the osteogenic differentiation from DPSCs, which may be useful for translational studies using DPSCs for bone tissue engineering.

Materials and methods

Cell culture. All procedures in the present study involving human participants were approved by the Ethics Committee of the Affiliated Hospital of Nantong University (Nantong, China), and performed according to the 1964 Helsinki declaration and its later amendments or comparable ethical standards. To obtain the DPSCs, normal human impacted third molars were first collected from 6 patients (age range: 22-41 years; 3 male, 3 female) with no carious lesions and oral infection between May and Aug, 2016. Written informed consent was obtained from all participants. The fresh isolated teeth were subsequently washed and opened to reveal the pulp chamber. A solution with 3 mg/ml collagenase type I was used to digest the pulp cells at 37˚C for 1 h. Single cell suspensions were obtained and cultured with Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin, in a humidified atmosphere containing 5% CO_2 and 95% air at 37˚C.

Osteogenic differentiation. Fresh DPSCs were cultured for three passages, prior to being used in the differentiation assay. In total, $2x10^4$ cells/cm^2 were cultured in DMEM supplemented with 0.1 mM dexamethasone, 10 mM β-glycerophosphate (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), 50 mg/ml ascorbic acid (Sigma-Aldrich; Merck KGaA) and 10 ng/ml TNF-α. DPSCs were differentiated for 7 or 14 days prior to being subjected to RNA extraction.

To transfect DPSCs at Day 1, control small interfering (si)RNA (5'-ACGUGACACGUUCGGAGAA-3'; 200 nM) and XIST siRNA (GCTTCTAACTAGCCTGAAT; 200 nM) were mixed with Lipofectamine® RNAiMAX Reagent (1:1; cat. no. 13778030; Thermo Fisher Scientific, Inc.) in opti-Minimum Essential Medium (Gibco; Thermo Fisher Scientific, Inc.); the solution was subsequently suspended in the culture DMEM. The culture medium was changed 16 h after transfection.

RNA extraction, RNA-seq and bioinformatics analysis. Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and assessed using the Agilent 2200 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, USA) for RNA quality. Samples were processed using the Illumina mRNA-Seq Sample Preparation kit (Illumina, Inc. San Diego, CA, USA; cat. nos. 1004824 and 1004825). RNA-seq libraries were 100 bp, paired-end sequenced on an Illumina HiSeq 2000. Sequencing reads following the removal of polymers, primer adaptors and ribosomal RNAs were aligned to the human genome with SOAPaligner/soap2 (version 2.21) (http://soap.genomics.org.cn/soapaligner.html). The alignment data was utilized to calculate the distribution of reads on reference genes and perform coverage analysis. The expression levels of individual RNAs were measured by reads per kilo-base per million following quality controls. Fisher's exact test was used for pathway enrichment analysis and gene-act-network analysis. A false discovery rate <0.05 was considered statistically significant. Kyoto Encyclopedia of Genes and Genomes analysis (KEGG; https://www.genome.jp/kegg/) was performed to identify specific pathways involved. The lncRNA expression pattern clustering was based on a previously published algorithm (18). To perform the coding non-coding co-expression network analysis, Pearson's correlation was calculated and the significant correlation pairs (>0.999) were selected, which were used to construct the network (19).

Table I. Primers for reverse transcription-quantitative polymerase chain reaction.

Gene	Sequence (5'-3')
MKLN1-F	CGCGGAGGACAACTTTTAGC
MKLN1-R	TTAGCTCCTTGCCTCGTTCC
SH3BP5-F	ATCAGGCTCAGGTTTGCTCC
SH3BP5-R	AGTCTCCTGTTCTCTTGATCAGC
THAP9-F	CGATGCGGAGATAATGGGGA
THAP9-R	TCCTTCCCTGCATATTTTGAGTAA
XIST-F	CCCTCATCCCCACTTTTCCC
XIST-R	TGGAATGAGCAGTGTGCGAT
GAPDH-F	AGAAGGCTGGGGCTCATTTG
GAPDH-R	AGGGGCCATCCACAGTCTTC

F, forward; R, reverse; MKLN1-AS1, MKLN1 antisense RNA 1; THAP9-AS1, THAP9 antisense RNA 1; SH3BP5-AS1, SH3BP5 antisense RNA 1; XIST, X inactive specific transcript.

RT-qPCR. Total RNA was extracted using TRIzol® reagent (Invitrogen, USA). RT was performed with the high-capacity cDNA RT kit (Applied Biosystems; Thermo Fisher Scientific, Inc.): 25˚C for 10 min, 37˚C for 120 min, 85˚C for 5 min and then held at 4˚C. Real-time PCR was performed in triplicate using SYBR green qPCR master mix (Qiagen GmbH, Hilden, Germany) and the CFX96 qPCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA): 95˚C for 5 min, 40 cycles of 95˚C, 15 sec, 60˚C 30 sec, 72˚C 30 sec, and 72˚C 10 min. The expression levels of GAPDH mRNA abundance were used for normalization and $2^{-\Delta\Delta Cq}$ method for quantification (20). Primers used for validation are listed in Table I.

Alkaline phosphatase (ALP) staining. Negative control or siRNA treated culture cells were fixed (4% paraformaldehyde in 1X PBS, room temperature for 2 h) and stained with the ALP assay kit (JianCheng, Nanjing, China) according to the manufacturer's instructions as previously described (8). Cultures were imaged were imaged with a Zeiss Apotome microscope (magnification, 20x) equipped with a Zeiss Axiocam MRM REV2 camera (Zeiss AG, Oberkochen, Germany). Cell counting was performed by eye.

Statistical analysis. For all figures, data are presented as the mean ± standard error of the mean, and the number (n) of samples used is indicated in the legends. Student's t-test and one-way analysis of variance with Bonferroni's correction for multiple comparisons (Prism 7.0, GraphPad Software, Inc., La Jolla, CA, USA) were performed to determine the significant differences between different groups. P<0.05 was considered to indicate a statistically significant difference.

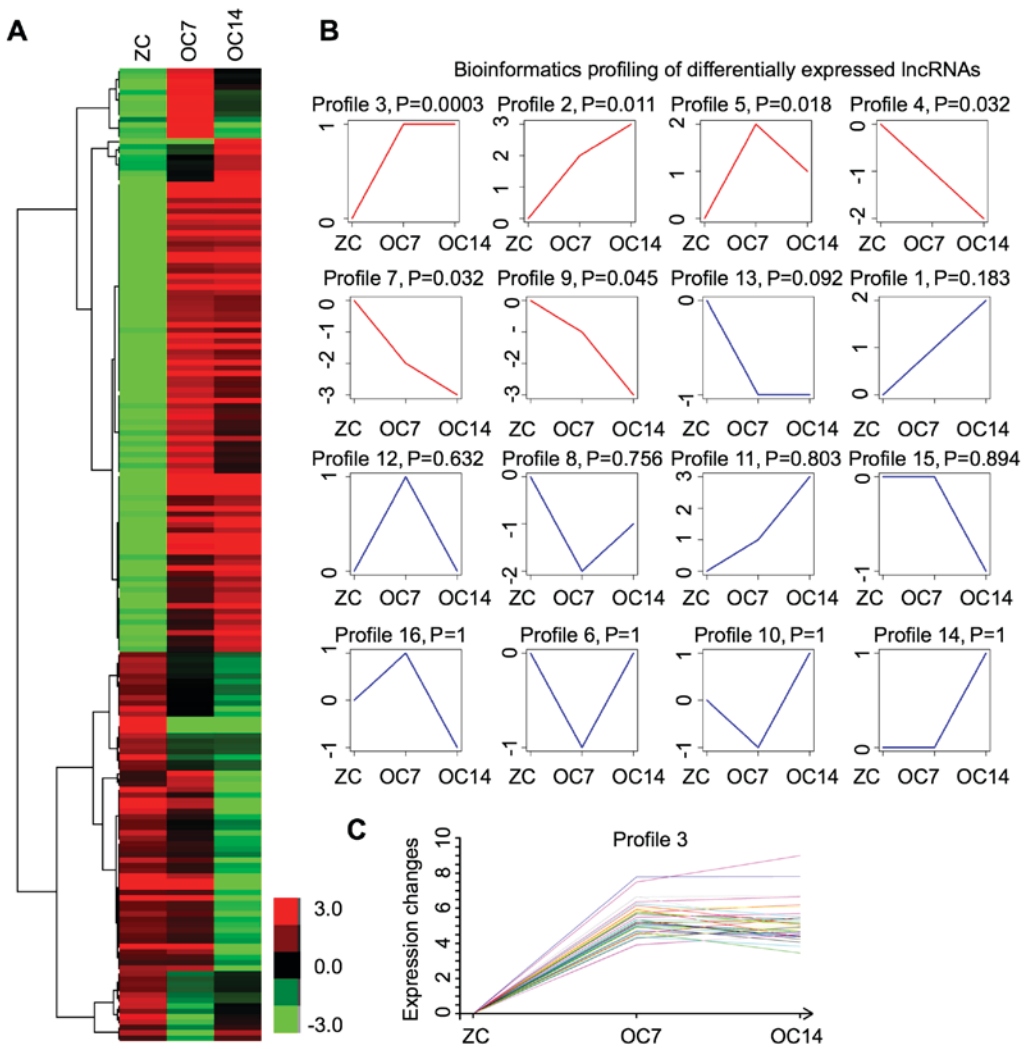

Figure 1. Profiling analysis of lncRNAs expression alterations during osteogenic differentiation of DPSCs. (A) Cluster analysis of lncRNAs that were significantly upregulated or downregulated at 7 and 14 days post tumor necrosis factor-α induction. Red indicate downregulated lncRNAs and green indicates upregulated lncRNAs. (B) Bioinformatics analysis of lncRNA expression pattern profiling. Profiles marked in red demonstrated statistical significance and profiles marked in blue were not significant. (C) Expression alterations of 34 lncRNAs in Profile 3 during the process of osteogenic differentiation of DPSCs. lncRNAs, long non-coding RNAs; DPSCs, dental pulp stem cells; ZC, undifferentiated PDSCs; OC7, osteogenic differentiation at 7 days; OC14, osteogenic differentiation at 14 days.

Results

Alterations of lncRNA expression during osteogenic differentiation of DPSCs. As previously demonstrated, DPSCs were treated using an osteogenic differentiation medium containing 10 ng/ml TNF-α (7,8). In the present study, RNA was collected after 7 and 14 days of treatment with TNF-α, when DPSCs were undergoing and completing osteogenic differentiation (8). The next generation sequencing analysis identified 77 and 133 lncRNAs, with 30 lncRNAs overlapping, which were differentially expressed at days 7 and 14 post-treatment, respectively (Fig. 1A). In addition, 58 and 73 were upregulated, and 19 and 60 were downregulated at day 7 and 14, respectively. These results demonstrated that lncRNAs underwent transitional alterations during osteogenic differentiation of DPSCs.

Profiling of differentially expressed lncRNAs. Subsequently, expression pattern analysis was performed on the differentially expressed lncRNAs during osteogenic differentiation of DPSCs. The bioinformatics analysis identified 16 different profiles (Fig. 1B). Among them, six demonstrated statistical significance (Fig. 1B). Subsequently, Profile 3 was analyzed for the following reasons: i) This profile demonstrated the highest statistical significance and ii) notably, the expression levels of all lncRNAs within this profile were increased at day 7 and their expression was maintained at relatively high expression levels until day 14 post-treatment with TNF-α (Fig. 1C).

To further validate the RNA-Seq results, the expression alterations of four predicted lncRNAs in Profile 3 were measured at 7 and 14 days post TNF-α induction by RT-qPCR. The results demonstrated a concomitant increase of all four lncRNAs at day 7, with different expression alterations at day 14 post-TNF-α induction, which was consistent with RNA-Seq data (Fig. 2).

Association between differentially expressed lncRNAs and mRNAs during osteogenic differentiation of DPSCs. In a previous study (8), mRNA alterations at day 7 and 14 post

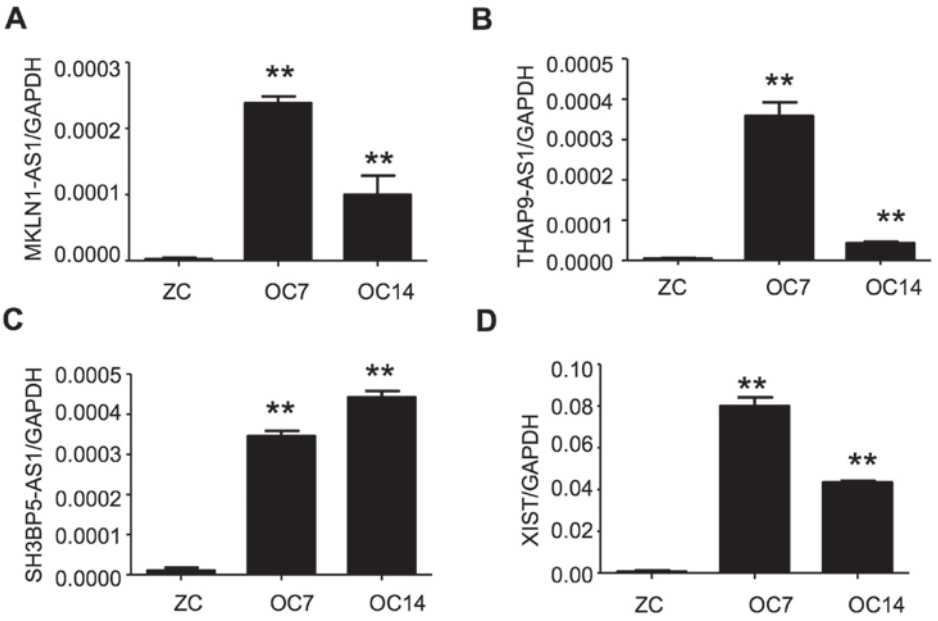

Figure 2. Validation of key lncRNAs demonstrating upregulation during osteogenic differentiation of dental pulp stem cells. Expression alterations at day 7 and 14 following tumor necrosis factor-α induction of selected (A) MKLN1-AS1, (B) THAP9-AS1, (C) SH3BP5-AS1 and (D) XIST in Profile 3 were validated by quantitative polymerase chain reaction. The GAPDH mRNA expression level was used for normalization. n=3 in each condition. **P<0.01 vs. ZC. lncRNAs, long non-coding RNAs; MKLN1-AS1, MKLN1 antisense RNA 1; THAP9-AS1, THAP9 antisense RNA 1; SH3BP5-AS1, SH3BP5 antisense RNA 1; XIST, X inactive specific transcript; ZC, undifferentiated PDSCs; OC7, osteogenic differentiation at 7 days; OC14, osteogenic differentiation at 14 days.

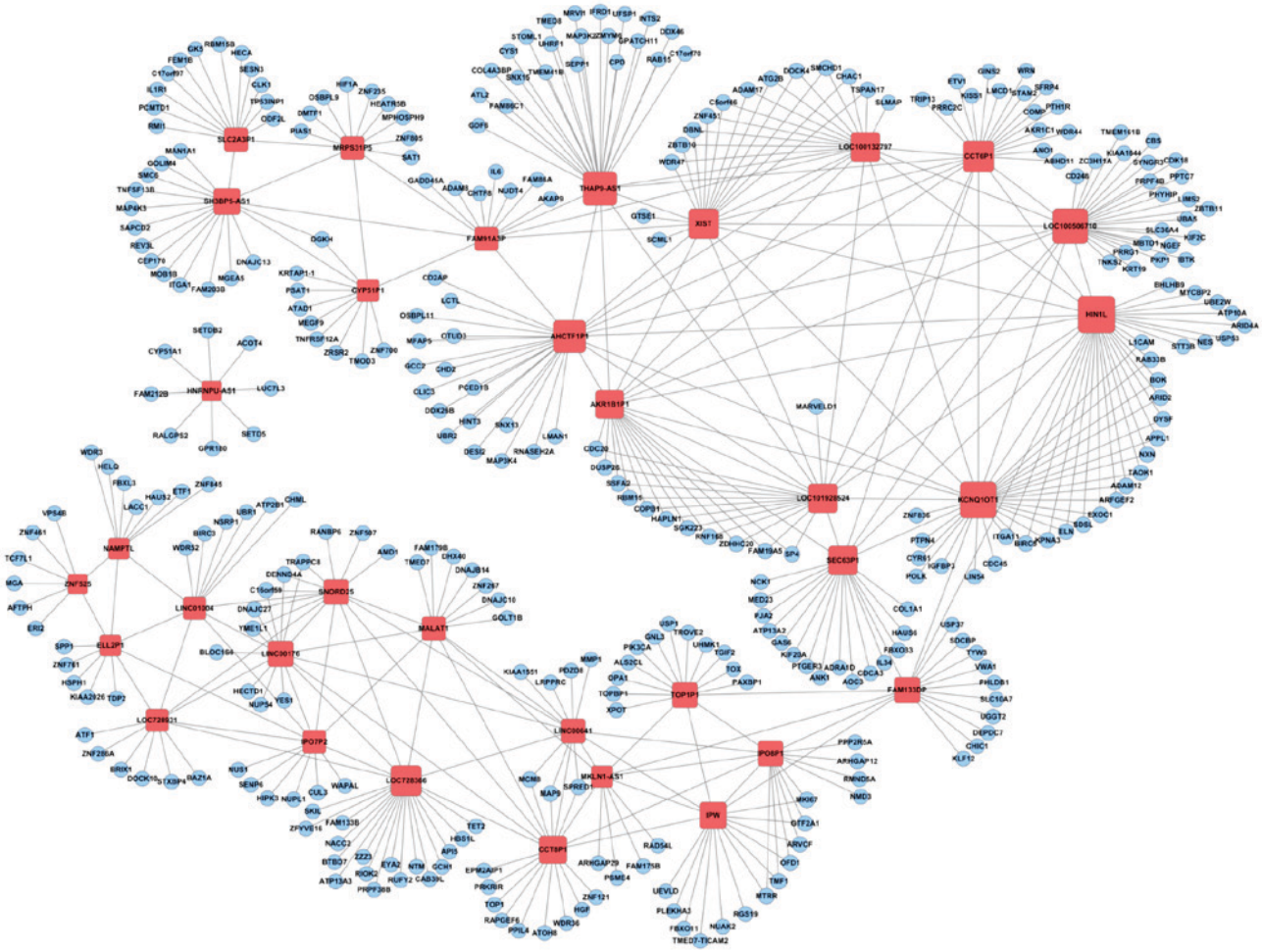

Figure 3. Co-expression network analysis of genes associated with key lncRNAs during osteoblast differentiation of DPSCs. Co-expression network analysis identified 336 mRNA transcripts associated with the 34 lncRNAs in Profile 3 during the process of osteogenic differentiation of DPSCs. Blue circles indicate mRNA transcripts and red squares indicate lncRNAs. lncRNAs, long non-coding RNAs; DPSCs, dental pulp stem cells.

Table II. Characterization of functional relevance to osteoblast differentiation in genes associated with key lncRNAs.

KEGG ID	Pathway	Count	P-value
04510	Focal adhesion	8	$8.48x10^5$
00260	Glycine, serine and threonine metabolism	4	0.0003
04512	ECM-receptor interaction	5	0.0005
03008	Ribosome biogenesis in eukaryotes	5	0.0005
04151	PI3K-Akt signaling pathway	9	0.0006
04141	Protein processing in endoplasmic reticulum	6	0.001
04115	p53 signaling pathway	4	0.002
04120	Ubiquitin mediated proteolysis	5	0.004
00270	Cysteine and methionine metabolism	3	0.007
04010	MAPK signaling pathway	6	0.012

KEGG analysis of the 336 mRNA transcripts associated with the 34 lncRNAs in Profile 3 during the process of osteogenic differentiation of dental pulp stem cells. KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long non-coding RNA; ECM, extracellular matrix; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; p53, cellular tumor antigen p53; MAPK, mitogen-activated protein kinase.

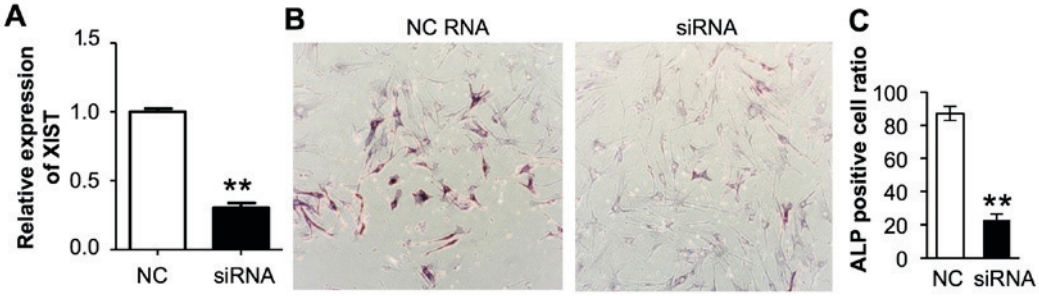

Figure 4. Effects of XIST knockdown on osteogenic differentiation of DSCPs. (A) Effects of XIST knockdown by control or XIST siRNA in DSCPs. The GAPDH mRNA expression level was used for normalization. **P<0.01, n=3,3 for control and XIST siRNA treated groups. (B) Representative images (magnification, x20) of control or XIST siRNA transfected DSCPs were stained for ALP at 14 days post culture with (C) quantification. **P<0.01, Student's t test. n=4,6 for control and XIST siRNA treated groups. XIST, X inactive specific transcript; DSCPs, dental pulp stem cells; siRNA, small interfering RNA; ALP, alkaline phosphatase; NC, negative control.

TNF-α induction were observed. Therefore, the present study aimed to investigate how lncRNAs alterations were associated with mRNA alterations during osteogenic differentiation of DPSCs. By bioinformatics analysis, 34 lncRNAs were predicted to be associated with 336 mRNA transcripts that underwent significant alterations during osteogenic differentiation (Fig. 3). KEGG analysis identified the 'PI3K-Akt signaling pathway' and 'MAPK signaling pathway', which have key roles in osteoblast differentiation (21-23) (Table II).

lncRNA XIST is required for efficient osteogenic differentiation of DPSCs induced by TNF-α. At present, whether and how lncRNAs are involved in osteoblast differentiation remains unclear. To investigate this, a validated lncRNA, XIST (Fig. 2), was selected and it was examined to determine how it may affect osteoblast differentiation. RT-qPCR confirmed that a specific siRNA was able to downregulate XIST expression in DPSCs (Fig. 4A). A total of 14 days after TNF-α induction, inhibition of XIST by siRNA in primarily cultured DPSCs significantly decreased the presence of alkaline phosphatase positive osteoblast cells (P<0.01; Fig. 4B and C). Therefore, XIST, an lncRNA is required for efficient osteogenic differentiation, possibly through a regulatory role in a group of mRNAs associated with this process.

Discussion

DPSCs are considered as ideal candidates for osteogenic differentiation, although their underlying mechanisms remain largely unknown. In the present study, the expression alterations of lncRNAs in TNF-α induced osteogenic differentiation of DPSCs were investigated. The present results identified transitional, global alterations of lncRNAs, which were associated with mRNAs involved in key signaling pathways for osteoblast differentiation. The present data further suggested that one lncRNA in particular, XIST, is essential for efficient osteogenic differentiation.

lncRNAs, as a type of non-coding RNAs, exhibit a variety of different cytotopic localizations and functional regulating modes. Such diversity and flexibility make lncRNAs good candidates to regulate gene expression in a temporospatial manner responding to complex situations, including during cellular differentiation (10-14). For example, multiple lncRNAs were demonstrated to serve important roles in neural, skin and muscle stem cell differentiation (24).

XIST encodes a 17-kb long non-coding RNA. Silencing factors are commonly recruited for global gene silencing on X chromosome (25). However, its role outside the X chromosome and during osteogenic differentiation is largely unknown. The aim of future research is to identify XIST-associated mRNA transcripts and to study their expressional alterations during TNF-α induced osteogenic differentiation of DPSCs. Due to the limitation of the current study, the contributions of many lncRNAs were not examined in TNF-α induced osteogenic differentiation. Nevertheless, the present findings provide insight for the understanding of molecular mechanisms underlying differentiation approaches of DPSCs and may be used to promote the potential regenerative therapies that use DPSCs as tissue resources.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

RT and YXL conceived and coordinated the study, designed, performed and analyzed the experiments, and wrote the paper. YKL, FL and ZYZ conducted the data collection and data analysis, and revised the paper. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All procedures performed in the present study involving human participants were approved by the Ethics Committee of the Affiliated Hospital of Nantong University (Nantong, China), and according to the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG and Shi S: Stem cell properties of human dental pulp stem cells. J Dent Res 81: 531-535, 2002.
2. Gronthos S, Mankani M, Brahim J, Robey PG and Shi S: Postnatal human dental pulp stem cells (DPSCs) in vitro and in. Proc Natl Acad Sci USA 97: 13625-13630, 2000.
3. Tatullo M, Marrelli M, Shakesheff KM and White LJ: Dental pulp stem cells: Function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med 9: 1205-1216, 2015.
4. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A and Papaccio G: Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: A pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14: 1162-1171, 2007.
5. Graziano A, d'Aquino R, Laino G and Papaccio G: Dental pulp stem cells: A promising tool for bone regeneration. Stem Cell Rev 4: 21-26, 2008.
6. Lindroos B, Mäenpää K, Ylikomi T, Oja H, Suuronen R and Miettinen S: Characterisation of human dental stem cells and buccal mucosa fibroblasts. Biochem Biophys Res Commun 368: 329-335, 2008.
7. Feng X, Feng G, Xing J, Shen B, Li L, Tan W, Xu Y, Liu S, Liu H, Jiang J, *et al*: TNF-α triggers osteogenic differentiation of human dental pulp stem cells via the NF-kappaB signalling pathway. Cell Biol Int 37: 1267-1275, 2013.
8. Liu YK, Zhou ZY and Liu F: Transcriptome changes during TNF-α promoted osteogenic differentiation of dental pulp stem cells (DPSCs). Biochem Biophys Res Commun 476: 426-430, 2016.
9. Gangaraju VK and Lin H: MicroRNAs: Key regulators of stem cells. Nat Rev Mol Cell Biol 10: 116-125, 2009.
10. Fatica A and Bozzoni I: Long non-coding RNAs: New players in cell differentiation and development. Nat Rev Genet 15: 7-21, 2014.
11. Luo S, Lu JY, Liu L, Yin Y, Chen C, Han X, Wu B, Xu R, Liu W, Yan P, *et al*: Divergent lncRNAs regulate gene expression and lineage differentiation in pluripotent cells. Cell Stem Cell 18: 637-652, 2016.
12. Geisler S and Coller J: RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 14: 699-712, 2013.
13. Guttman M and Rinn JL: Modular regulatory principles of large non-coding RNAs. Nature 482: 339-346, 2012.
14. Engreitz JM, Ollikainen N and Guttman M: Long non-coding RNAs: Spatial amplifiers that control nuclear structure and gene expression. Nat Rev Mol Cell Biol 17: 756-770, 2016.
15. Huang Y, Zheng Y, Jia L and Li W: Long noncoding RNA H19 promotes osteoblast differentiation via TGF-β1/Smad3/HDAC signaling pathway by deriving miR-675. Stem Cells 33: 3481-3492, 2015.
16. Li H, Zhang Z, Chen Z and Zhang D: Osteogenic growth peptide promotes osteogenic differentiation of mesenchymal stem cells mediated by LncRNA AK141205-induced upregulation of CXCL13. Biochem Biophys Res Commun 466: 82-88, 2015.
17. Zhuang W, Ge X, Yang S, Huang M, Zhuang W, Chen P, Zhang X, Fu J, Qu J and Li B: Upregulation of lncRNA MEG3 promotes osteogenic differentiation of mesenchymal stem cells from multiple myeloma patients by targeting BMP4 transcription. Stem Cells 33: 1985-1997, 2015.
18. Ramoni MF, Sebastiani P and Kohane IS: Cluster analysis of gene expression dynamics. Proc Natl Acad Sci USA 99: 9121-9126, 2002.
19. Shi J, Chen X, Li H, Wu Y, Wang S, Shi W, Chen J and Ni Y: Neuron-autonomous transcriptome changes upon ischemia/reperfusion injury. Sci Rep 7: 5800, 2017.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
21. James AW: Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. Scientifica (Cairo) 2013: 684736, 2013.
22. Ge C, Xiao G, Jiang D and Franceschi RT: Critical role of the extracellular signal-regulated kinase-MAPK pathway in osteoblast differentiation and skeletal development. J Cell Biol 176: 709-718, 2007.
23. Rahman MS, Akhtar N, Jamil HM, Banik RS and Asaduzzaman SM: TGF-β/BMP signaling and other molecular events: Regulation of osteoblastogenesis and bone formation. Bone Res 3: 15005, 2015.
24. Flynn RA and Chang HY: Long noncoding RNAs in cell-fate programming and reprogramming. Cell Stem Cell 14: 752-761, 2014.
25. Cerase A, Pintacuda G, Tattermusch A and Avner P: Xist localization and function: New insights from multiple levels. Genome Biol 16: 166, 2015.