Biochemical and Biophysical Research Communications 456 (2015) 459–464

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Long noncoding RNAs expression signatures in chondrogenic differentiation of human bone marrow mesenchymal stem cells

Liang Wang, Zhengyao Li, Ziquan Li, Bin Yu, Yipeng Wang *

Department of Orthopedic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Shuaifuyuan Hutong, Beijing 100730, China

ARTICLE INFO

Article history:
Received 24 November 2014
Available online 4 December 2014

Keywords:
Mesenchymal stem cell
Bone marrow
Long noncoding RNA
Differentiation
Chondrogenesis

ABSTRACT

Long non-coding RNAs (lncRNAs) have been established to participate in various biological processes that are crucial for development and differentiation. However, the roles of lncRNAs in the mechanisms of human bone marrow mesenchymal stem cells (MSCs) differentiation are not completely understood. The purpose of the study was to investigate the expression profiles of lncRNAs during the chondrogenic differentiation of human bone marrow MSCs, with a view to studying the biological function of lncRNAs and their involvement in the mechanism of differentiation. We compared the lncRNAs expression profiles of undifferentiated and differentiated cells during chondrogenic differentiation by microarray. 3638 differentially expressed lncRNAs were identified (fold-change >2.0 or <−2.0, $P < 0.05$), consisting of 2166 up-regulated and 1472 down-regulated. Microarray data were validated using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Bioinformatic analyses were applied for further study of these differentially expressed lncRNAs. Among these lncRNAs, ZBED3-AS1 and CTA-941F9.9 were further analyzed with co-expression network and target prediction analysis. The results showed that the two up-regulated lncRNAs are likely to play important roles in chondrogenic differentiation process. In conclusion, the expression profile of lncRNAs was significantly altered during differentiation process. It provided a new insight on complicated regulation mechanisms of human bone marrow MSCs chondrogenic differentiation.

1. Introduction

Mesenchymal stem cells (MSCs) are capable of self-renewal and can be differentiated into the osteogenic, chondrogenic, and adipogenic lineages. They are a source of cells in bone and cartilage tissue engineering [1]. Human bone marrow MSCs have advantages of availability, culture expansion, low immunogenic properties [2], and ease of genetic manipulation, so they have wide use in numerous clinical applications, including tissue engineering [3], autoimmune disease [4], myocardial infarction treatment [5] and wound repair [6]. The differentiation processes involve complex pathways regulated at both transcriptional and posttranscriptional levels. However, the precise molecular mechanisms of differentiation remain largely unknown.

Long noncoding RNAs (lncRNAs) belong to a novel heterogeneous class of ncRNAs that includes thousands of different species. lncRNAs have crucial roles in gene expression control during both developmental and differentiation processes. Unlike small ncRNAs, lncRNAs can fold into complex secondary and higher order structures to provide greater potential and versatility for both protein and target recognition [7]. They have been found to play crucial regulatory roles in a diverse range of biological pathways and cellular processes, including genomic imprinting, chromosome inactivation [8], differentiation [9] and carcinogenesis [10], in transcriptional, posttranscriptional and even translational levels [11]. Until now, the potential role for lncRNAs in the chondrogenic differentiation of human bone marrow MSCs remains unknown.

In this study, we obtained differential expression profiles of lncRNAs and mRNAs of undifferentiated versus chondro-differentiated human bone marrow MSCs. The putative functions and target genes of these lncRNAs were predicted using bioinformatic analysis. Our observations demonstrated that lncRNAs expression profiles are likely to provide important insights into mechanism of differentiation of MSCs.

2. Materials and methods

2.1. Cell culture

Human bone marrow mesenchymal stem cells (MSCs) were purchased from ScienCell Research Laboratories. Cells were

* Corresponding author. Fax: +86 10 69152809.
E-mail address: wlliangwang@sina.com (Y. Wang).

http://dx.doi.org/10.1016/j.bbrc.2014.11.106
0006-291X/

cultured following the instructions recommended by the manufacturer. The MSCs were expanded in Mesenchymal Stem Cell Medium (MSCM, ScienCell) at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. The confluent cells were transferred to next passage using 0.25% trypsin for up to three passages.

2.2. Chondrogenic differentiation of MSCs

For chondrogenic differentiation, the pellet culture was performed for three-dimensional culture. Approximately 1×10^6 cells (passage 3) were placed in a 15 ml polypropylene tube and centrifuged at 500*g* for 5 min. The cells were cultured in Mesenchymal stem cell Chondrogenic Differentiation medium (MCDM, ScienCell), in the presence of 10 ng/mL recombinant human TGF-β3. The cell pellets formed free-floating aggregates within the first 24 h. The medium was replaced every 3 days. The cells on day 0 were undifferentiated and regarded as control. Three biological replicates were performed for cultured cells during differentiation. Aggregates were harvested for RNA extraction at day 0, 7, 14, 21 and 28 after induction.

2.3. Analysis of MSCs differentiation

Chondrogenic differentiation was established by histological staining using Alcian blue and immunohistological staining of Aggrecan, Sox-9 and Collagen II (Col II). qRT-PCR analysis of expression levels of chondrogenic marker genes were performed at day 14, including Aggrecan, Sox-9 and Col II.

Alcian blue staining was utilized to examine cartilage nodules. The cells were fixed in 4% paraformaldehyde for 30 min, followed by staining with alcian blue for 10 min. After dehydrated, the stained cells were then photographed.

For the immunohistochemical staining, the pellets to be processed for staining were routinely fixed by 4% paraformaldehyde, dehydrated and paraffin imbedded. 5 μm sections were stained by 0.1% toluidine blue for 2 min. Sections were immunohistochemistry stained for type II collagen, aggrecan and Sox-9 using rabbit polyclonal antibody. Following deparafinization, rehydration, antigen retrieval under boil, nonspecific antibody binding blocked. Sections were incubated with primary antibody (1:200 diluted) overnight at 4 °C and followed to be rinsed with PBS. Sections were then incubated with biotin-conjugated anti-rabbit IgG secondary antibody (1:200 diluted) for 60 min. The antibody biotin conjugates were detected with streptavidin–biotin-horseradish peroxidase complex applied for 30 min using diaminobenzidine as substrate.

2.4. RNA extraction

Total RNA was extracted using Trizol Reagent (Invitrogen) according to the instructions recommended by the manufacture. The RNA purity and concentration was evaluated with Nano Drop ND-1000 spectrophotometer. RNA integrity was determined with 1% formaldehyde denaturing gel electrophoresis, which revealed a good quality.

2.5. Microarray analysis

Briefly, three undifferentiated cell samples at day 0 were regarded as control group and three chondro-differentiated cell samples at day 14 were regarded as experiment group. These three-paired samples were used to synthesize double-stranded complementary DNA (cDNA) and the cDNA was labeled and hybridized to lncRNA + mRNA Human Gene Expression Microarray V4.0 (CapitalBio Corp, Beijing, China) according to the manufacturer's instructions.

The data from lncRNA + mRNA microarray were used to analyze data summarization, normalization and quality control using the GeneSpring software V11.5 (Agilent). The differentially expressed genes were selected if the change of threshold values were >2.0 or <−2.0 folds and if Benjamini–Hochberg corrected *P* values were <0.05. The data was normalized and hierarchically clustered with CLUSTER 3.0 software. The data were performed tree visualization with Java Treeview software (Stanford University School of Medicine, Stanford, CA, USA).

2.6. GO and pathway analysis

GO analysis was derived from Gene Ontology (www.geneontology.org), which provides three structured networks of defined terms that describe gene product attributes. The *P* value denotes the significance of GO Term enrichment in the differentially expressed mRNA list ($P < 0.05$ was considered statistically significant). We also performed pathway analysis for the differentially expressed mRNAs based on the latest KEGG (Kyoto Encyclopedia of Genes and Genomes) database. This analysis allowed us to determine the biological pathways for which a significant enrichment of differentially expressed mRNAs existed ($P < 0.05$ was considered statistically significant).

2.7. Construction of the coding-non-coding gene co-expression (CNC) network

The CNC network was constructed based on the correlation analysis between the differentially expressed lncRNAs and mRNAs.

Table 1
Primers used for qRT-PCR.

Genes	Sense primer (5′–3′)	Anti-sense primer (3′–5′)
Aggrecan	CCCAAGAATCAAGTGGAGCCG	ACACGATGCCTTTCACCACGA
Sox-9	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
Col II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
GAPDH	ATTTGGTCGTATTGGGCG	TGGAAGATGGTGATGGGATT
CILP	CCAGGCTGGGGAGTACTTTTGC	TTATGCCCACCTGGGTGCTCAG
FOSL1	AACCGGAGGAAGGAACTGAC	CTGCAGCCCAGATTTCTCA
RGS4	GGCTTCTTGCTTGAGGAGTG	TCCAGTGATTCAGCCCATTTC
SCRG1	TTGCTCTGCTGCCCAAAAGAC	GAAATCAGGAATGGTGTTCTCCAG
WISP1	AGGTATGGCAGAGGTGCAAG	GTGTGTGTAGGCAGGGAGTG
ENST00000433576.1	ACACTGTGGGACTTCTTAGCC	GTTTCTGGTGCTGGTGTCTT
CTA-941F9.9	TGCCCAAGCCTCCTAATG	AATACGGCCTCTATCTGACG
XLOC_008374	ACTATGAGACTGGACTGGAACA	AGGTGAGTGGGAAGGAGC
LINC00707	CACTATGAGACTGGACTGGAACA	TGGTCTTGGGAGTGGTGC
ZBED3-AS1	TACAACTTTGCATTAACCTTCC	TGCCCTGTCCTCATGTTCG

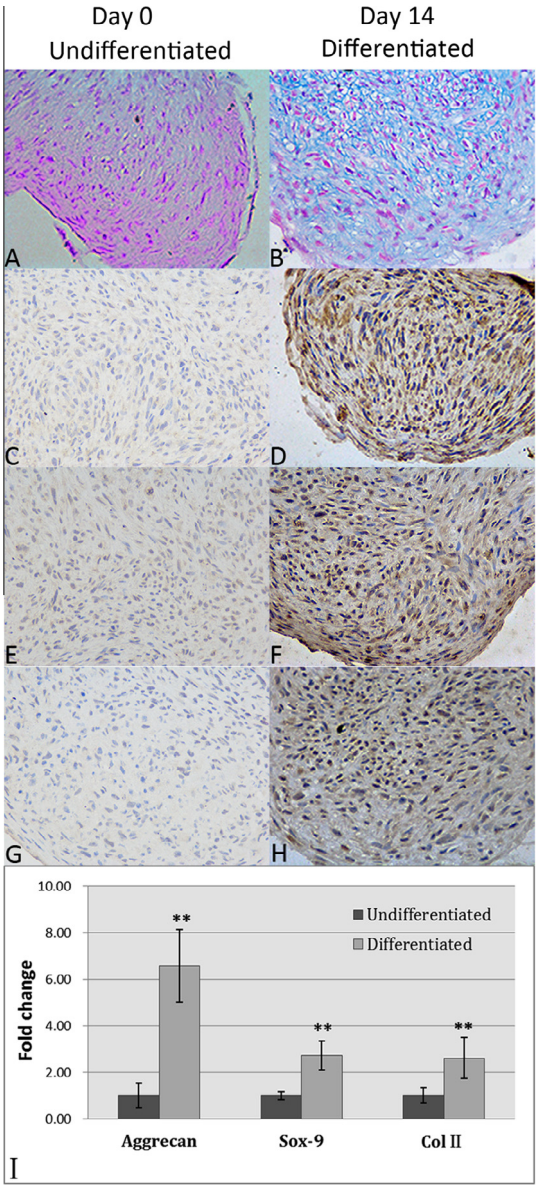

Fig. 1. Chondrogenic differentiation of human bone marrow MSCs. (A and B) Alcian blue staining (×400). (C and D) Immunohistochemical staining of Aggrecan (×400). (E and F) Immunohistochemical staining of Col II (×400). (G and H) Immunohistochemical staining of Sox-9 (×400). (I) Chondrogenic differentiation was confirmed by qRT-PCR analysis of genes of Aggrecan, Sox-9 and Col II at day 14. The relative expression levels were normalized to day 0 control values, to represent the relative fold change in expression. **The change of expression levels was significant with $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

LncRNAs and mRNAs with Pearson correlation coefficients not less than 0.99 were selected to draw the network using open source bioinformatics software Cytoscape (Institute of Systems Biology in Seattle). In network analysis, yellow node represents the lncRNA and green node represents the mRNA. Violet lines indicate a positive correlation, and pink lines indicate an inverse correlation.

2.8. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RT-PCR was performed using SYBR Premix Ex Taq on Thermal Cycler Dice TP800 instrument. A total of 2 μg total RNA was used for cDNA synthesis with the PrimeScript RT Reagent Kit Perfect Real Time (TaKaRa Biotechnology) according to the manufacturer's

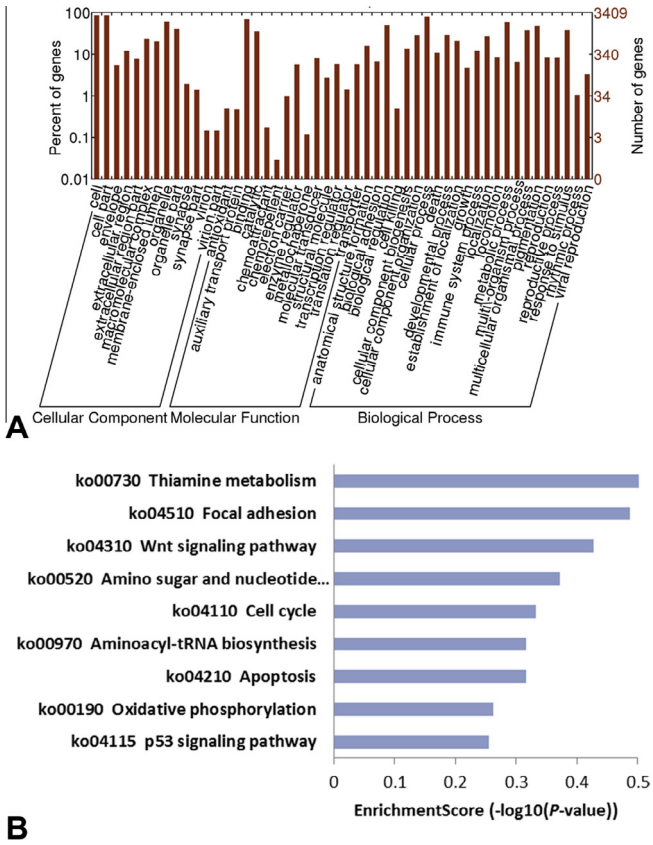

Fig. 2. Go (A) and pathway (B) analysis of mRNAs differentially expressed between undifferentiated and chondro-differentiated MSCs.

instructions. RT-PCR was performed in a 20 μl of reaction system, including 10 μl SYBR Premix Ex Taq (2×), 0.4 μl of PCR forward primer (10 μM), 0.4 μl of PCR reverse primer (10 μM), 1 μl of cDNA and 8.2 μl of double-distilled water. The qPCR reaction was set at an initial denaturation of 10 min at 95 °C, and followed by 95 °C (5 s), 52 °C (30 s), 72 °C (30 s) in a total 40 cycles and a final extension step at 72 °C for 5 min. All experiments were performed in triplicate. For each sample, GAPDH expression was analyzed to normalize target gene expression. Relative gene expression was calculated using $2^{-\triangle\triangle Ct}$ method. Primer sequences used in this study are shown in Table 1.

2.9. Statistical analysis

All assays were repeated with a minimum of 3 times. Data were analyzed using SPSS 19.0 software. All quantitative data were expressed as mean ± standard deviation (SD). Gene expression levels in chondrogenic differentiation lineage were compared between undifferentiated and differentiated samples. The Student's *t*-test was used to compare data between the two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Expression profiles of lncRNAs during chondrogenic differentiation

Chondrogenic differentiation of MSCs was induced by using standard chondrogenic-induction medium (MCDM). Both histological staining using Alcian Blue and immunohistochemical staining of Aggrecan, Sox-9and Col II showed that the micromass pellets had differentiated toward the chondrogenic lineage. qRT-PCR

Table 2
The characteristics of lncRNAs for validation.

lncRNA	Expression	Chromosome	Strand	Start	End	Class
ZBED3-AS1	Up	Chr 5	+	76,382,838	76,444,336	Antisense
CTA-941F9.9	Up	Chr 22	–	46,000,311	46,001,501	Intergenic
ENST00000433576.1	Up	Chr 1	–	222,054,322	222,092,054	Intergenic
LINC00707	Down	Chr 10	+	6,821,559	6,884,868	Intergenic
XLOC_008374	Down	Chr 10	+	6,875,609	6,882,671	Intergenic

detected dramatic increase in chondrocyte-specific genes expression in chondrogenic differentiation at day 14 after induction, including Aggrecan, Sox-9 and Col II (Fig. 1).

The lncRNAs expression profiles during chondrogenic differentiation were identified for MSCs 14 d after chondrogenic induction using lncRNA microarray. Expression levels of lncRNAs were significantly altered between differentiated and non-differentiated cells. 3638 differentially expressed lncRNAs were identified (fold-change >2.0 or <−2.0, $P < 0.05$). Among these, 2166 lncRNAs were found to be up-regulated more than 2-fold in the chondro-differentiated cells compared to undifferentiated cells, while 1472 lncRNAs were down-regulated more than 2-fold ($P < 0.05$). When the cut-off was set at 5-fold, 374 lncRNAs were up-regulated and 163 were down-regulated ($P < 0.05$).

3.2. Expression profiles of mRNAs during differentiation

The expression profiles of protein-coding RNAs were also performed with mRNAs microarray. 5560 mRNAs were found to be differentially expressed, including 1980 that were up-regulated more than 2-fold and 3580 that were down-regulated more than 2-fold ($P < 0.05$). When the cut-off was set at 5-fold, 480 lncRNAs were up-regulated and 377 were down-regulated ($P < 0.05$).

GO analysis showed that the most significant biological processes consisted of cellular metabolic process, cellular component organization or biogenesis, response to stimulus, regulation of developmental process, regulation of signal transduction. Pathway analysis showed that the most significant pathways consisted of p53 signaling pathway, Wnt signaling pathway, Apoptosis and Cell cycle (Fig. 2).

3.3. qRT-PCR analysis of lncRNAs and mRNAs expression

According to fold difference, gene locus, and so on, we initially identified a number of candidate lncRNAs and mRNAs for validation. Five lncRNAs (ZBED3-AS1, CTA-941F9.9, LINC00707, ENST00000433576.1 and XLOC_008374) and five mRNAs (CILP, FOSL1, RGS4, SCRG1 and WISP1) were further examined using qPCR. The characteristics of these lncRNAs and mRNAs were shown in Table 2. The results of qRT-PCR and microarray were consistent (Fig. 3).

3.4. LncRNA classification and subgroup analysis

Analysis of the genomic context of lncRNAs that were expressed during differentiation could help in predicting their functional role. Therefore, we analyzed the association of lncRNAs and mRNAs to identify putative functional relationships. We categorized the relationship between lncRNAs and mRNAs as sense, antisense, intronic, bidirectional, or intergenic. Among differentiated expressed lncRNAs associated with chondro-differentiation, 330 sense, 472 antisense, 267 intronic, 212 bidirectional, and 701 intergenic ncRNAs were identified, respectively.

In addition, lncRNAs with enhancer-like function (lncRNA-a) were identified using GENCODE annotation. The expression profiles of 231 enhancer-like lncRNAs indicated that they were differentially expressed (fold-change >2.0 or <−2.0, $P < 0.05$) between

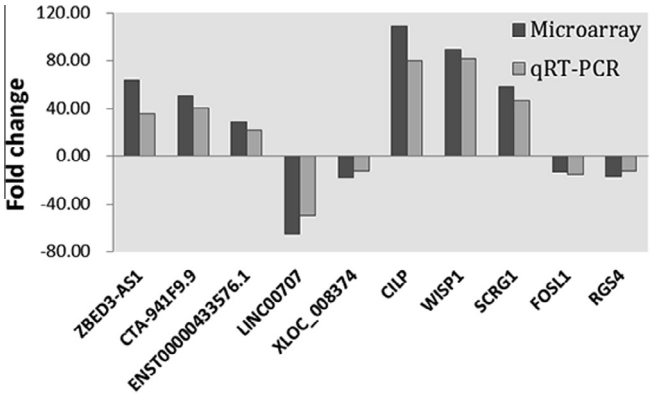

Fig. 3. Comparison between microarray data and qRT-PCR result for lncRNAs and mRNAs. The validation results indicated that the microarray data correlated well with the qPCR results.

undifferentiated and differentiated cells. Among these, 156 were up-regulated and 75 were down-regulated. Then, a class of ~3500 large intergenic ncRNAs (lincRNAs) were also identified. In all, 40 lincRNAs, including 25 up-regulated and 15 down-regulated were observed (fold-change >2.0 or <−2.0, $P < 0.05$).

3.5. Construction of the lncRNA-mRNA co-expression network

In order to ascertain the correlation between differentially expressed lncRNA and mRNA, we constructed lncRNA-mRNA co-expression network for differential expressed lncRNAs. The network indicates that one mRNA is correlated with one to tens of lncRNAs and vice versa. Thus, we proposed that the expression profile of mRNAs and lncRNAs are significantly correlated.

In addition, we observed the dynamic expression trend of two lncRNAs (ZBED3-AS1, CTA-941F9.9) during the chondrogenic differentiation process. The expression levels at day 0, 7, 14, 21 and 28 were compared using qRT-PCR (Fig. 4). The trend showed

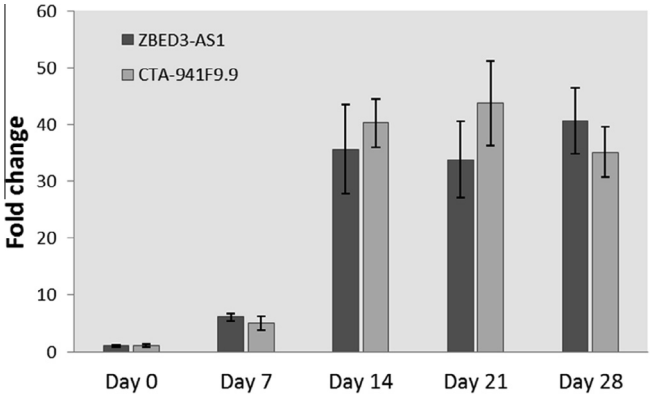

Fig. 4. The dynamic expression trends of two lncRNAs (ZBED3-AS1 and CTA-941F9.9) during the chondrogenic differentiation process of human bone marrow MSCs. The trend showed that the expressions of two lncRNAs at day 7 were significant higher than day 0. The expressions continued to rise to higher levels at day 14 and the levels maintained until day 28.

that the expressions of two lncRNAs at day 7 were significant higher than day 0. The expressions continued to rise to higher levels at day 14 and the levels maintained until day 28.

4. Discussion

Stem cells are present in a variety of mesenchymal tissues other than bone marrow and can be isolated from them, such as skeletal muscle [12], trabecular bone [13], synovium [14], adipose tissue [15], umbilical cord blood [16] and periosteum [17]. Human bone marrow MSCs derive from mesodermal cell lineages with self-renewable capacities and multi-directional differentiation potentials [18,19]. Their ease of isolation and culture and their multilineage differentiation potential make MSCs suitable and appealing cells in tissue engineering. Given appropriate culture conditions, BMSCs have been reported to differentiate into chondrocytes [3,20], osteocytes [21], adipocytes [22], endothelial cells [23], cardiomyocytes [24], and even hepatocytes [25] and neurons [26]. MSCs can differentiate into into chondrocytes to form cartilage during the initial stages of endochondral ossification [27].

The lncRNAs are evolutionarily conserved non-coding RNAs that are longer than 200 nucleo tides in length and lacking of protein-encoding capacity. The ratio of lncRNAs in total ncRNAs is beyond 80% but is the least well-understood [28,29]. Although initially thought to be transcriptional noise, recent evidence suggests that the expression of lncRNAs is cell- and developmental stage-specific and regulated by common transcription factors [30,31]. LncRNAs are abundantly encoded in mammalian genomes, numbering in the tens of thousands. However, functionalizing the rich repertoire of long non-protein-coding transcripts remains a challenge.

An understanding of the transcriptional regulatory circuitry that is responsible for pluripotency and differentiation in human MSCs is fundamental to understanding molecular mechanism and realizing the therapeutic potential of the stem cells. Although the functional roles of miRNAs in chondrogenesis of MSC have been extensively investigated [32–34], the role of lncRNAs in this regulatory circuitry and their underlying mechanism remains undefined.

It has been demonstrated that the some lncRNAs express differently during cell development and lineage commitment [35]. Some researches focused on the regulation of pluripotency and differentiation in stem cells. Guttman et al. performed loss-of-function studies on lincRNAs expressed in mouse embryonic stem cells (ESCs) and characterized the effects on gene expression [9]. The results demonstrate that lincRNAs have key roles in the circuitry controlling cell state. lincRNAs are key components of the ESCs transcriptional network that are functionally important for maintaining the pluripotent state, and that many are down-regulated upon differentiation. Sheik et al. established conserved lncRNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells [36].

For human stem cells, Loewer et al. has identified numerous lincRNAs with expression linked to pluripotency, these include linc-RoR, which serves as a critical regulator of cellular reprogramming of human induced pluripotent stem cells [37]. Wang et al. revealed that the expression of linc-RoR serves as endogenous miRNA sponge and regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal [38]. All these studies demonstrated there is growing evidence that lncRNAs have a critical role in the biology of various stem cell types. However, the role of lncRNAs in the human mesenchymal stem cells derived from bone marrow has not been studied.

Our study first demonstrated lncRNAs and mRNAs expression profiles during the chondrogenic differentiation of human bone marrow MSCs using lncRNAs and mRNAs microarray. We subsequently validated the microarray results by qRT-PCR. Bioinformatic analyses were applied for further study of these differentially expressed lncRNAs and mRNAs, including GO, Pathway and co-expression network analysis.

During chondrogenic differentiation, we selected five lncRNAs for qPCR analysis. Furthermore, we predicted the target genes of two lncRNAs (ZBED3-AS1 and CTA-941F9.9) by bioinformatics and constructed co-expression network. In the co-expression network, the two lncRNAs correlated with other lncRNAs and mRNAs and some mRNAs was related with cell chondrogenic differentiation. In addition, the expression trends of two lncRNAs were observed during the differentiation process. The expressions of ZBED3-AS1 and CTA-941F9.9 significantly increased from day 7, peaked at day 14 and maintained to day 28. The trends may suggest that the two lncRNAs may play roles in early stage of chondrogenic differentiation. Of course, the further related researches will be needed to validate the hypothesis.

In summary, to our knowledge the first time, we have shown the global different expression profiles of lncRNAs during the chondrogenic differentiation of human bone marrow MSCs. By bioinformatics prediction, we obtained some target genes correlated with the process of differentiation of the candidate lncRNAs. Further studies in function analysis of these lncRNAs are needed to provide more conclusive evidence to explain the regulatory mechanisms. Collectively, our results suggest that lncRNAs should be the important regulators in the course of chondrogenic differentiation and might provide a guide for further investigation about the mechanisms of lncRNAs regulating differentiation process of human bone marrow MSCs.

Acknowledgment

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 81372007).

References

[1] J.E. Frith, B. Thomson, P.G. Genever, Dynamic three-dimensional culture methods enhance mesenchymal stem cell properties and increase therapeutic potential, Tissue Eng. Part C Methods 16 (2010) 735–749.
[2] F. Rastegar, D. Shenaq, J. Huang, W. Zhang, B.Q. Zhang, B.C. He, L. Chen, G.W. Zuo, Q. Luo, Q. Shi, E.R. Wagner, E. Huang, Y. Gao, J.L. Gao, S.H. Kim, J.Z. Zhou, Y. Bi, Y. Su, G. Zhu, J. Luo, X. Luo, J. Qin, R.R. Reid, H.H. Luu, R.C. Haydon, Z.L. Deng, T.C. He, Mesenchymal stem cells: molecular characteristics and clinical applications, World J. Stem Cells 2 (2010) 67–80.
[3] P. Macchiarini, P. Jungebluth, T. Go, M.A. Asnaghi, L.E. Rees, T.A. Cogan, A. Dodson, J. Martorell, S. Bellini, P.P. Parnigotto, S.C. Dickinson, A.P. Hollander, S. Mantero, M.T. Conconi, M.A. Birchall, Clinical transplantation of a tissue-engineered airway, Lancet 372 (2008) 2023–2030.
[4] M.E. Reinders, J.W. de Fijter, H. Roelofs, I.M. Bajema, D.K. de Vries, A.F. Schaapherder, F.H. Claas, P.P. van Miert, D.L. Roelen, C. vanKooten, W.E. Fibbe, T.J. Rabelink, Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study, Stem Cells Transl. Med. 2 (2013) 107–111.
[5] A.R. Williams, K.E. Hatzistergos, B. Addicott, F. McCall, D. Carvalho, V. Suncion, A.R. Morales, S.J. Da, M.A. Sussman, A.W. Heldman, J.M. Hare, Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction, Circulation 127 (2013) 213–223.
[6] P. Liu, Z. Deng, S. Han, T. Liu, N. Wen, W. Lu, X. Geng, S. Huang, Y. Jin, Tissue-engineered skin containing mesenchymal stem cells improves burn wounds, Artif. Organs 32 (2008) 925–931.
[7] M. Guttman, J.L. Rinn, Modular regulatory principles of large non-coding RNAs, Nature 482 (2012) 339–346.
[8] J.T. Lee, M.S. Bartolomei, X-inactivation, imprinting, and long noncoding RNAs in health and disease, Cell 152 (2013) 1308–1323.
[9] M. Guttman, J. Donaghey, B.W. Carey, M. Garber, J.K. Grenier, G. Munson, G. Young, A.B. Lucas, R. Ach, L. Bruhn, X. Yang, I. Amit, A. Meissner, A. Regev, J.L. Rinn, D.E. Root, E.S. Lander, LincRNAs act in the circuitry controlling pluripotency and differentiation, Nature 477 (2011) 295–300.
[10] R. Spizzo, M.I. Almeida, A. Colombatti, G.A. Calin, Long non-coding RNAs and cancer: a new frontier of translational research, Oncogene 31 (2012) 4577–4587.
[11] X. Li, Z. Wu, X. Fu, W. Han, Long noncoding RNAs: insights from biological features and functions to diseases, Med. Res. Rev. 33 (2013) 517–553.

[12] E.M. Horwitz, P.L. Gordon, W.K. Koo, J.C. Marx, M.D. Neel, R.Y. McNall, L. Muul, T. Hofmann, Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 8932–8937.
[13] Y. Sakaguchi, I. Sekiya, K. Yagishita, S. Ichinose, K. Shinomiya, T. Muneta, Suspended cells from trabecular bone by collagenase digestion become virtually identical to mesenchymal stem cells obtained from marrow aspirates, Blood 104 (2004) 2728–2735.
[14] C. De Bari, F. Dell'Accio, P. Tylzanowski, F.P. Luyten, Multipotent mesenchymal stem cells from adult human synovial membrane, Arthritis Rheum. 44 (2001) 1928–1942.
[15] P.A. Zuk, M. Zhu, P. Ashjian, D.A. De Ugarte, J.I. Huang, H. Mizuno, Z.C. Alfonso, J.K. Fraser, P. Benhaim, M.H. Hedrick, Human adipose tissue is a source of multipotent stem cells, Mol. Biol. Cell. 13 (2002) 4279–4295.
[16] O.K. Lee, T.K. Kuo, W.M. Chen, K.D. Lee, S.L. Hsieh, T.H. Chen, Isolation of multipotent mesenchymal stem cells from umbilical cord blood, Blood 103 (2004) 1669–1675.
[17] T. Fukumoto, J.W. Sperling, A. Sanyal, J.S. Fitzsimmons, G.G. Reinholz, C.A. Conover, S.W. O'Driscoll, Combined effects of insulin-like growth factor-1 and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis in vitro, Osteoarthritis Cartilage 11 (2003) 55–64.
[18] P. Bianco, P.G. Robey, Stem cells in tissue engineering, Nature 414 (2001) 118–121.
[19] E. Jones, D. McGonagle, Human bone marrow mesenchymal stem cells in vivo, Rheumatology (Oxford) 47 (2008) 126–131.
[20] P. Jungebluth, E. Alici, S. Baiguera, B.K. Le, P. Blomberg, B. Bozoky, C. Crowley, O. Einarsson, K.H. Grinnemo, T. Gudbjartsson, G.S. Le, G. Henriksson, O. Hermanson, J.E. Juto, B. Leidner, T. Lilja, J. Liska, T. Luedde, V. Lundin, G. Moll, B. Nilsson, C. Roderburg, S. Stromblad, T. Sutlu, A.I. Teixeira, E. Watz, A. Seifalian, P. Macchiarini, Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study, Lancet 378 (2011) 1997–2004.
[21] T.J. Heino, T.A. Hentunen, Differentiation of osteoblasts and osteocytes from mesenchymal stem cells, Curr. Stem Cell Res. Ther. 3 (2008) 131–145.
[22] M. Karagianni, I. Brinkmann, S. Kinzebach, M. Grassl, C. Weiss, P. Bugert, K. Bieback, A comparative analysis of the adipogenic potential in human mesenchymal stromal cells from cord blood and other sources, Cytotherapy 15 (2013) 76–88.
[23] M. Crisan, Transition of mesenchymal stem/stromal cells to endothelial cells, Stem Cell Res. Ther. 4 (2013) 95.
[24] J.W. Zhao, M.R. Zhang, Q.Y. Ji, F.J. Xing, L.J. Meng, Y. Wang, The role of slingshot-1L (SSH1L) in the differentiation of human bone marrow mesenchymal stem cells into cardiomyocyte-like cells, Molecules 17 (2012) 14975–14994.
[25] S. Pulavendran, M. Rajam, C. Rose, A.B. Mandal, Hepatocyte growth factor incorporated chitosan nanoparticles differentiate murine bone marrow mesenchymal stem cell into hepatocytes in vitro, IET Nanobiotechnol. 4 (2010) 51–60.
[26] P. Mohammad-Gharibani, T. Tiraihi, S.A. Mesbah-Namin, J. Arabkheradmand, H. Kazemi, Induction of bone marrow stromal cells into GABAergic neuronal phenotype using creatine as inducer, Restor. Neurol. Neurosci. 30 (2012) 511–525.
[27] A. Augello, C. De Bari, The regulation of differentiation in mesenchymal stem cells, Hum. Gene Ther. 21 (2010) 1226–1238.
[28] C.P. Ponting, P.L. Oliver, W. Reik, Evolution and functions of long noncoding RNAs, Cell 136 (2009) 629–641.
[29] E.A. Gibb, C.J. Brown, W.L. Lam, The functional role of long non-coding RNA in human carcinomas, Mol. Cancer 10 (2011) 38.
[30] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159.
[31] M.C. Tsai, O. Manor, Y. Wan, N. Mosammaparast, J.K. Wang, F. Lan, Y. Shi, E. Segal, H.Y. Chang, Long noncoding RNA as modular scaffold of histone modification complexes, Science 329 (2010) 689–693.
[32] Y. Zhang, R.L. Xie, J. Gordon, K. LeBlanc, J.L. Stein, J.B. Lian, A.J. van Wijnen, G.S. Stein, Control of mesenchymal lineage progression by microRNAs targeting skeletal gene regulators Trps1 and Runx2, J. Biol. Chem. 287 (2012) 21926–21935.
[33] M. Weber, A.M. Sotoca, P. Kupfer, R. Guthke, E.J. van Zoelen, Dynamic modelling of microRNA regulation during mesenchymal stem cell differentiation, BMC Syst. Biol. 7 (2013) 124.
[34] V.K. Gangaraju, H. Lin, MicroRNAs: key regulators of stem cells, Nat. Rev. Mol. Cell Biol. 10 (2009) 116–125.
[35] J.T. Kung, D. Colognori, J.T. Lee, Long noncoding RNAs: past, present, and future, Genetics 193 (2013) 651–669.
[36] M.J. Sheik, P.M. Gaughwin, B. Lim, P. Robson, L. Lipovich, Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells, RNA 16 (2010) 324–337.
[37] S. Loewer, M.N. Cabili, M. Guttman, Y.H. Loh, K. Thomas, I.H. Park, M. Garber, M. Curran, T. Onder, S. Agarwal, P.D. Manos, S. Datta, E.S. Lander, T.M. Schlaeger, G.Q. Daley, J.L. Rinn, Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells, Nat. Genet. 42 (2010) 1113–1117.
[38] Y. Wang, Z. Xu, J. Jiang, C. Xu, J. Kang, L. Xiao, M. Wu, J. Xiong, X. Guo, H. Liu, Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal, Dev. Cell 25 (2013) 69–80.