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Identification of long non-coding RNA involved in osteogenic differentiation from mesenchymal stem cells using RNA-Seq data

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ABSTRACT. The aim of this study was to identify long non-coding RNA (lncRNA) associated with osteogenic differentiation from mesenchymal stem cells (MSCs) using high-throughput RNA sequencing (RNA-Seq) data. RNA-Seq dataset was obtained from the European Bioinformatics Institute (accession No. PRJEB4496), including two replicates each for immortalized mesenchymal stem cells iMSC#3 cultured in growth medium (GM) and differentiation medium (DM) for 28 days. The clean reads were aligned to a hg19 reference genome by Tophat and assembled by Cufflinks to identify the known and novel transcripts. RPKM values were calculated to screen for differentially expressed RNA. Novel lncRNA were screened based on various filter criteria. Subsequently, the underlying function of novel lncRNAs were predicted by functional annotation by ERPIN, a co-expression network was constructed by WGCNA and the KEGG pathway enriched by KOBAS. A total of 3171 RNA differentially expressed between the DM and GM groups (2597 mRNA and 574 lncRNA) were identified. Among the 574 differentially expressed lncRNA, 357 were known and 217 were novel lncRNA. Furthermore, 32 novel lncRNA were found to be miRNA precursors (including miR-689, miR-640, miR-601, and miR-544). A total of

14,275 co-expression relationships and 217 co-expression networks were obtained between novel lncRNA and mRNA. The differentially expressed lncRNA and mRNA were enriched into 6 significant pathways, including those for cancer, ECM-receptor interaction, and focal adhesion. Therefore, novel lncRNAwere identified and their underlying function predicted, which may provide the basis for future analyses of the role of lncRNA in osteoblastic differentiation.

Key words: Long non-coding RNA; Osteogenic differentiation; Mesenchymal stem cells

INTRODUCTION

Mesenchymal stem cells (MSCs) are a population of stromal cells present in the bone marrow and most connective tissues, capable of self-renewal and differentiation into multiple cell lineage, e.g., myoblasts, adipocytes, chondrocytes, and osteoblasts (Nombela-Arrieta et al., 2011). The differentiation of MSCs into specific cell types is elaborately regulated by a serial of signaling cascades (Augello and De Bari, 2010). The activation and overexpression of adenosine receptor A2BR induced the differentiation of MSCs into osteoblasts during the early stages; however, A2AR was highly expressed during the later stages of osteoblastic differentiation (Gharibiet al., 2011). Interleukin-1β (IL-1β) effectively and rapidly induces human MSC differentiation into osteoblasts through the non-canonical Wnt-5a/ receptor tyrosine kinase-like orphan receptor 2 (Ror2) pathway (Sonomotoet al., 2012). BMP-2 functions as an effective osteoblast-inducing signal and can trigger osteoblastic cell differentiation alone, or in combination with other molecules (Zhang et al., 2008; Lin et al., 2014). In addition, microRNA (miRNA) have also been implicated in osteoblast differentiation. For example, Zeng et al. (2012) reported that the down-regulation of miR-100 promotes osteogenic differentiation of adipose-derived MSCs *in vitro* by up-regulating bone morphogenetic protein receptor type II (BMPR2) expression. On the other hand, Deng et al. (2013) demonstrated that the inhibition of miR-31 dramatically increased the alkaline phosphatase activity, mineralization, and expression of osteogenic transcription factors OPN, BSP, OSX, and OCN in bone MSC cultures, ultimately contributing to osteogenic differentiation (Deng, 2013). However, the molecular mechanisms of osteoblast differentiation remain to be understood.

Long non-coding RNA (lncRNA) are a recently discovered class of non-coding functional RNA that are believed to be key regulators of diverse biological processes, including cell differentiation. Zhu and Xu (2013) demonstrated that reduced lncRNA ANCR expression caused osteoblast differentiation, whereas ANCR overexpression inhibited osteoblast differentiation. Further studies targeting enhancer of zeste homolog 2 (EZH2) and regulating Runx2 expression have indicated that ANCR may be associated with osteoblast differentiation (Zhu and Xu, 2013). Zuo et al. (2013) utilized the Arraystar lncRNA array to screen for lncRNA related with early osteoblast differentiation of C3H10T1/2 MSCs, and thus identified 116 differentially expressed lncRNA (Zuo, 2013). However, very few groups have focused on lncRNA. lncRNA and their underlying roles remain to be further investigated.

The goal of this study was to identify the lncRNA expression in MSCs differentiating into osteoblasts by not using RNA-sequencing data (Sun et al., 2012; Ren et al., 2012). Differentially expressed, novel lncRNAs were then selected for function prediction using several bioinformatics analyses. Our findings may provide a theoretical foundation for future studies on lncRNA modulation of osteoblastic differentiation.

MATERIAL AND METHODS

Datasets

The RNA-Seq dataset for osteogenic differentiationcan be obtained from the European Bioinformatics Institute (ENA) database using the accession number PRJEB4496 (http://www.ebi.ac.uk/ena/data/view/PRJEB4496). This dataset included two replicates of immortalized mesenchymal stem cells iMSC#3, which were cultured in minimum essential medium alpha (MEM Alpha) growth medium (GM) supplemented with Glutamax (Life Technologies, Carlsbad, CA, USA) and 10% fetal calf serum (FCS; Integro, Zaandam, The Netherlands), and two replicates ofiMSC#3 cells, which were cultured indifferentiation medium [DM; GM supplemented with 67 mM ascorbic acid 2-phosphate, 3.5 mM b-glycerol phosphate, and 10 nM dexamethasone (all Sigma-Aldrich, St. Louis, MO)] for 28 days. The iMSC#3 GM group consisted of the experiment accession ERX302478 (run accession ERR329500) and ERX302479 (run accession ERR329502) while the iMSC#3 DM group contained theexperiment accession ERX302480 (run accession ERR329501) and ERX302481 (run accession ERR329499). RNA libraries were sequenced with the Illumina Genome Analyzer II system according to the manufacturer protocols (FC-104-5001; Illumina, San Diego, CA, USA), by performing a paired-end run with 75 bp read length for all samples (Hakelien et al., 2014) .

Raw read processing

The raw reads were processed by removing the adaptor sequences (overlap = 5) using CutAdapt (Martin, 2011), low quality sequences (< 10% 'N' bases and > 85% QA >20 bases) using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html), and ribosomal sequences with Tophat (Trapnell et al., 2009). All subsequent analyses were performed using clean reads.

Mapping reads to the human genome

Clean reads were aligned to the hg19 reference genome (downloaded from the UCSC website) using Tophat (Trapnell et al., 2009), during which only 2 mismatches and 2 gaps were allowed for each read. The mapped reads were then assembled using Cufflinks (Trapnell, 2010) to identify the known and novel transcripts.

Gene expression analysis

The number of reads mapped to each transcript was determined using the HT-Seq software (http://www-huber.embl.de/users/anders/HTSeq/doc/index.html) (Anders et al., 2014). Each gene expression level was measured by the number of uniquely mapped reads per kilobase of exon region in a gene per million mappable reads (RPKM)(Mortazavi et al., 2008). The longest transcript was selected to calculate the RPKM for genes with more than one alternative transcript. Replicates were compared with the input RNA sample using the edge R package (Robinson et al., 2010).

Novel lncRNA detection

We attempted to screen for novel lncRNA using the novel transcript, based on the following filter criteria: 1) length > 200 nucleotides (nt); 2) open reading frame (ORF) length <300 nt; 3) no

match to PFAM, the protein families database (Sammut et al., 2008) (E value >1e-5); 4) iSeeRNA (Sun, 2013) non-coding scoreL > 0.5; and 5) the Coding Potential Assessment Tool (CPAT) (Wang et al., 2013) coding probability > 0.375.

Novel lncRNA classification

The predicted functional classification of a lncRNA was performed using the Infernal (INFERence of RNA ALignment) (http://infernal.janelia.org) software platform (Nawrocki and Eddy, 2013), and the Rfam database (ftp://ftp.sanger.ac.uk/pub/databases/Rfam/CURRENT/) (Burge et al., 2013). Rfam is a database built from structure-annotated multiple sequence alignments using covariance models (CMs) and family annotations for a number of non-coding RNA families. The Infernal software implements the covariance models, and therefore acts as a core for the building process of and search strategy in Rfam.

Functional annotation of novel lncRNA

ANNOVAR (http://www.openbioinformatics.org/annovar/)(Wang et al., 2010) is a command-line tool that uses information from the UCSC Genome Browser to provide annotations. ANNOVAR was used to annotate the novel lncRNA and to find the corresponding sites relative to the reference gene. Blat (Kent, 2002) was then used to align the lncRNA and mRNA to screen for sequence homology. Furthermore, ERPIN (Lambert et al., 2004) was applied to search for miRNA precursors.

Co-expression between lncRNA and mRNA

The co-expression between lncRNA and mRNA was identified by Weighted Gene Co-expression Network Analysis (WGCNA) in the R software package (Langfelder and Horvath, 2008), according to the cut-off point of adjacency >0.98°; the co-expression network was constructed and visualized using theIgraph R package (Toshkov, 2012).

Functional enrichment of lncRNA and mRNA

All function enrichment analyses were performed using the KOBAS software (Xie et al., 2011) to identify the significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The corrected P value < 0.05 was set as the significance level.

RESULTS

Clean reads were obtained after theremoval of adaptor, low quality, and ribosomal sequences. As shown in Figure 1, the quality of base was obviously improved after filtering. Using the Tophat tool, we mapped an average of 98.46% (range: 98.3-98.56%) of the reads to the human genome (UCSC version hg19) (Table 1). By calculating the RPKM values and comparing the sequences of the GM group with those of the DM group, we screened 3171 differentially expressed RNAs, including 2597 mRNAs and 574 lncRNAs, according to the cut-off criteria of false discovery rate (FDR)< 0.05 and |logFC| > 1.

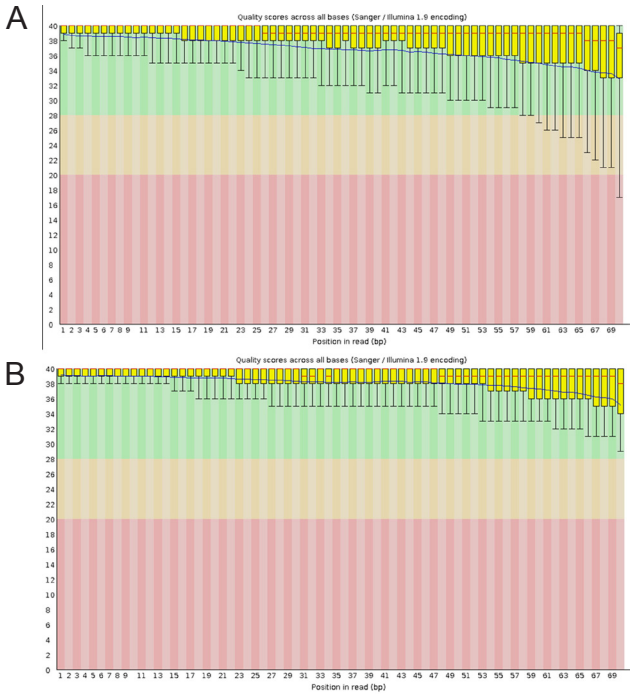

Figure 1. Quality control results of the ERR329499 sample. **A.** before the quality filter; **B.** after the quality filter.

Table 1. Read map results.

Mapped statistics	ERR329499 (N, %)	ERR329501 (N, %)	ERR329500 (N, %)	ERR329502 (n, %)
Effective reads	48,948,102 (100)	44,799,910 (100)	44,861,624 (100)	46,417,054 (100)
Total mapped	48,212,358 (98.50)	44,156,132 (98.56)	44,182,655 (98.49)	45,629,556 (98.30)
Multiple mapped	561,991 (1.15)	482,019 (1.08)	539,291 (1.20)	516,780 (1.11)
Uniquely mapped	47,650,367 (97.35)	43,674,113 (97.49)	43,643,364 (97.28)	45,112,776 (97.19)
Read1 mapped	24,118,187 (49.27)	22,090,050 (49.31)	22,102,503 (49.27)	22,828,640 (49.18)
Read2 mapped	24,094,171 (49.22)	22,066,082 (49.25)	22,080,152 (49.22)	22,800,916 (49.12)
Reads map to '+'	24,131,291 (49.30)	22,098,675 (49.33)	22,115,195 (49.30)	22,842,026 (49.21)
Reads map to '-'	24,081,067 (49.20)	22,057,457 (49.24)	22,067,460 (49.19)	22,787,530 (49.09)
Reads mapped in proper pairs	23,801,430 (48.63)	21,805,376 (48.67)	21,800,117 (48.59)	22,483,581 (48.44)
Duplication	10,887,772 (22.24)	9,405,838 (21.00)	7,529,155 (16.78)	8,576,247 (18.48)

Among the 574 differentially expressed lncRNA, Cufflinks alignment (which produced 1270 novel lncRNA) indicated that 357 were known lncRNA, while the remaining 217 were novel lncRNA (Figure 2). Furthermore, we also investigated the functional classification of novel lncRNA; four lncRNA families were taken from the Rfam database, including tRNA (RF00005), U1 (RF00003), Y_RNA (RF00019), and U3 (RF00012) (Table 2). TCONS_00046478, a novel differentially expressed lncRNA, belonged to the tRNA (RF00005) family. lncRNA generally exert a cis-acting regulatory effect on the mRNA of the same chromosome (Kornienko, 2013). Therefore, 100 kb upstream or downstream of novel lncRNA may be lncRNA-related genes; these were also screened as shown in Table 3. ERPIN tool was also used to predict the miRNA precursor. We discovered 32 novel lncRNA, especially 9 differentially expressed lncRNA with corresponding miRNA precursors, including miR-330, miR-689, miR-787, miR-675, miR-640, miR-601, and miR-544 (Table 4).

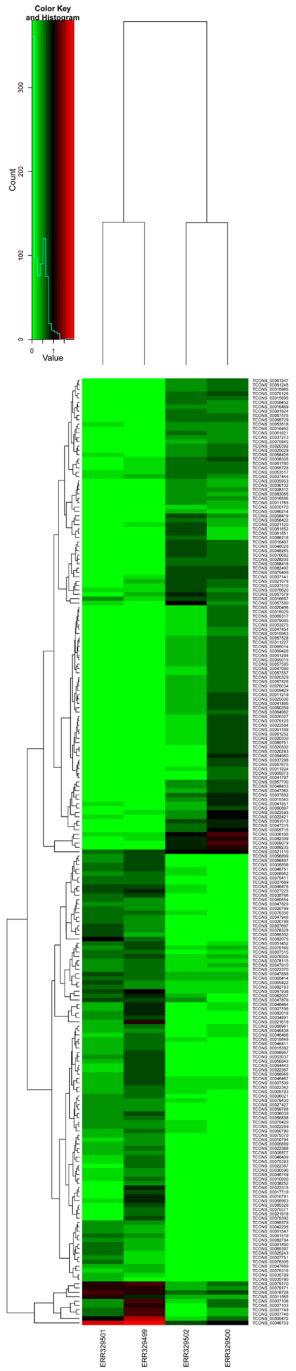

Figure 2. Heat Map of the differentially expressed, novel lncRNA. The horizontal axis indicates the sample name, while the vertical axis presents the name of the novel lncRNA. The heat map was constructed using the following formula: log10 (rpkm + 1).

Table 2. Rfam analysis results.

Transcript	Rfam annotation
TCONS_00035394	E-value = 5.71e-25; gc-content = 50; id = U3.1; model_end = 218; model_start = 1; rfam-acc = RF00012; rfam-id = U3
TCONS_00047573	E-value = 3.74e-19; gc-content = 42; id = Y_RNA.1; model_end = 96; model_start = 1; rfam-acc = RF00019; rfam-id = Y_RNA
TCONS_00007020	E-value = 6.70e-14; gc-content = 51; id = U1.1; model_end = 165; model_start = 1; rfam-acc = RF00003; rfam-id = U1
TCONS_00046478	E-value = 2.10e-09; gc-content = 57; id = tRNA.1; model_end = 71; model_start = 1; rfam-acc = RF00005; rfam-id = tRNA

Table 3. Relative situation between lncRNA and refGene.

New lncRNA	Gene	Distance
TCONS_00079142	*LINC00967*	9427
TCONS_00032275	*CCR7*	<1000
TCONS_00061531	*TSPAN5*	3412
TCONS_00085751	*IGSF1*	71759
TCONS_00060774	*MAD2L1*	<1000
TCONS_00007638	*MARCKSL1*	22003
TCONS_00007638	*FAM229A*	2235
TCONS_00016367	*GRIA4*	21555
TCONS_00016367	*MSANTD4*	3158

Table 4. miRNA precursor of lncRNA.

lncRNA	miRNA	E value	lncRNA	miRNA	E value
TCONS_00058390	*miR-370*	1.54E-03	**TCONS_00046411**	*miR-787*	2.59E-03
TCONS_00053037	*miR-330*	2.29E-03	**TCONS_00053517**	*miR-675*	1.37E-03
TCONS_00046611	*miR-689*	8.79E-06	TCONS_00008224	*miR-612*	1.78E-04
TCONS_00085813	*miR-689*	2.62E-05	TCONS_00035001	*miR-640*	4.67E-03
TCONS_00011565	*miR-689*	7.31E-06	TCONS_00035851	*miR-640*	4.67E-03
TCONS_00035472	*miR-689*	4.79E-05	TCONS_00083021	*miR-640*	1.91E-03
TCONS_00046478	*miR-689*	3.25E-04	TCONS_00058341	*miR-640*	5.02E-04
TCONS_00069054	*miR-689*	6.57E-05	**TCONS_00007697**	*miR-640*	1.24E-04
TCONS_00050573	*miR-689*	3.52E-04	TCONS_00058112	*miR-562*	3.08E-03
TCONS_00081035	*miR-689*	4.90E-04	**TCONS_00070170**	*miR-601*	3.90E-03
TCONS_00005788	*miR-689*	4.39E-04	TCONS_00069054	*miR-636*	2.31E-03
TCONS_00034926	*miR-689*	5.45E-05	TCONS_00042752	*miR-554*	2.14E-04
TCONS_00035851	*miR-689*	3.44E-07	TCONS_00008325	*miR-891*	2.85E-03
TCONS_00006252	*miR-689*	1.40E-05	**TCONS_00027225**	*miR-544*	4.75E-04
TCONS_00053517	*miR-689*	2.32E-04	TCONS_00061677	*miR-544*	3.77E-04
TCONS_00082563	*miR-689*	1.96E-05	TCONS_00043130	*miR-544*	1.78E-11

The bold lncRNA are differentially expressed, novel.

WGCNA was performed to explore the relationship between co-expressed, differentially-expressed lncRNA and mRNA. Based on the adjacency threshold of 0.98, 14275 co-expression relationships and 217 co-expression networks were obtained between novel lncRNA and mRNA. The co-expression networksof lncRNA TCONS_00046478, TCONS_00027225, and TCONS_00007697are shown in Figures 3-5. In addition, the underlying function of differentially expressed lncRNA and mRNA were predicted by KEGG pathway enrichment analysis. As shown in Table 5, six pathways were significantly enriched, including a cancer pathway (hsa05200), and pathways regulating ECM-receptor interaction (hsa04512), focal adhesion (hsa04510), pathogenic *Escherichia coli* infection (hsa05130), proteoglycans in cancer (hsa05205), and the Rap1 signaling pathway (hsa04015).

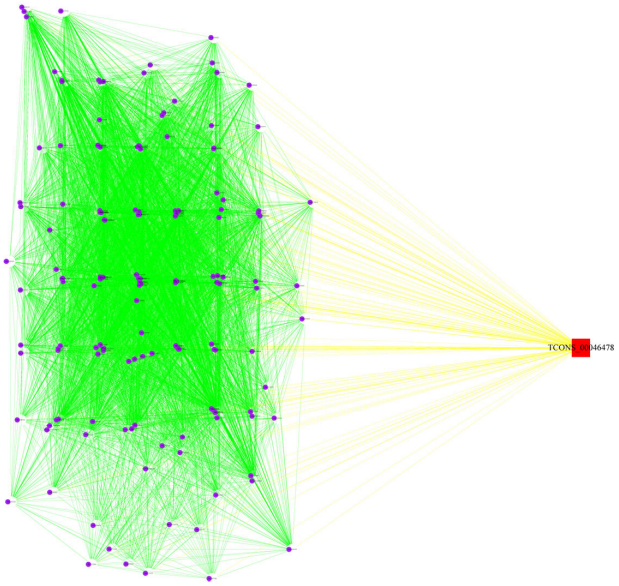

Figure 3. Co-expression network between lncRNA TCONS_00046478 and the refGene. The red box indicates novel lncRNA and the purple circle indicates the refGene; the yellow line expresses the co-expression relationship between lncRNA and mRNA; and the green line indicates the co-expression relationship between mRNAs.

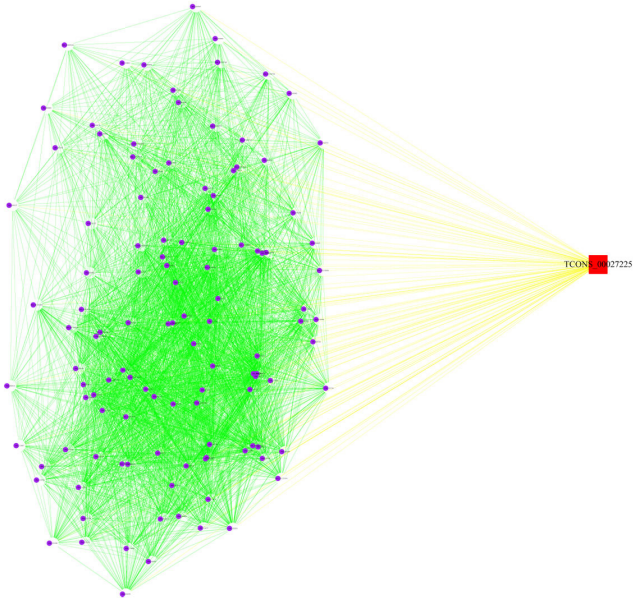

Figure 4. Co-expression network between lncRNA TCONS_00027225 and the refGene. The red box indicates novel lncRNA and the purple circle denotes refGene; the yellow line expresses the co-expression relationship between lncRNA and mRNA; and the green line denotes the co-expression relationship between mRNAs.

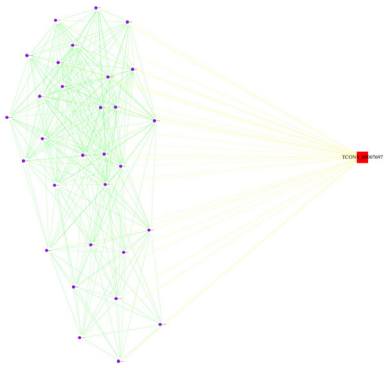

Figure 5. Co-expression network between lncRNA TCONS_00007697 and the refGene. The red box indicates novel lncRNA and the purple circle denotes refGene; the yellow line indicates the co-expression relationship between lncRNA and mRNA; and the green line denotes the co-expression relationship between mRNAs.

Table 5. KEGG pathway enrichment of lncRNA and mRNA.

Term	ID	Corrected P value	Enriched genes
Pathways in cancer	hsa05200	0.021894	CKS2; PDGFRB; GLI2; GLI3; CCND1; BCL2; SMAD3; RASSF5; LAMA1; AXIN2; LAMA2; PIK3R3; FZD7; BIRC5; HSP90AA1; PTGS2; PLCG1; TPM3; E2F2; FZD5; FZD8; ITGA6; FZD1; ITGB1; FZD6; ITGA3; LEF1; ARNT2; SLC2A1; STK4; CKS1B; IGF1; DVL2; COL4A4; WNT5B; CDK2; BRCA2; WNT2; WNT3; WNT5A; WNT7A; HGF; DAPK2; CSF1R; HHIP; LAMB3; RASSF1; LAMB1; CCNE2; CCNE1; IL6; CYCS; CDH1; FIGF; CDC42; MECOM; JUP; WNT10B; TGFB3; TGFB2; E2F1; CTNNB1; PDGFRA; MMP2; FGFR1; COL4A3; COL4A2; ZBTB16; FGF2; COL4A1; RUNX1; SOS1; STAT1; FOS; FOXO1; RAC2; FGF13; MAPK9; FGF10; WNT7B; HSP90B1; FGF7; FGF5
ECM-receptor interaction	hsa04512	0.021894	AGRN; COL11A1; LAMA1; LAMA2; ITGA5; LAMB3; ITGA6; HMMR; THBS2; THBS3; ITGB3; ITGB5; THBS1; ITGB8; COL6A2; COL6A3; ITGA3; ITGA10; LAMB1; COMP; COL24A1; ITGA11; ITGB1; COL3A1; COL4A3; COL4A2; COL4A4; CD44; COL4A1; COL5A1
Focal adhesion	hsa04510	0.021894	PDGFRB; PIK3R3; CCND1; COL11A1; BCL2; KDR; LAMA1; LAMA2; SHC3; PDGFRA; ACTB; ITGA5; LAMB3; MAPK9; ITGA6; ACTN4; ITGA3; THBS2; THBS3; PAK3; COL4A1; PXN; ITGB5; CAV2; IGF1; ITGB8; PDGFD; VCL; COL6A2; COL6A3; HGF; ITGA10; LAMB1; CCND3; COL24A1; ITGB1; CDC42; ITGA11; CAV1; CTNNB1; COMP; COL4A3; COL4A2; FIGF; COL4A4; COL3A1; FLNC; DIAPH1; FLNB; SOS1; COL5A1; ITGB3; RAC2; VASP; CAPN2; TLN2; THBS1
Pathogenic *Escherichia coli* infection	hsa05130	0.036856	NCK1; TUBB2A; TUBA1C; TUBB2B; TUBB6; CDH1; KRT18; CDC42; TLR4; ACTB; TUBB; TUBB4B; YWHAZ; TUBB3; CTNNB1; TUBA1B; EZR; LY96; ITGB1; WASL; TUBA4A
Proteoglycans in cancer	hsa05205	0.045938	PIK3R3; ITGB5; CCND1; KDR; THBS1; ACTB; MSN; ITGA5; PLCG1; FZD5; FZD8; FZD7; ESR1; FZD6; LUM; CAV1; DCN; PXN; FZD1; HBEGF; CAV2; IGF1; TLR4; EZR; WNT5B; WNT2; WNT3; WNT5A; WNT7A; HGF; GPC1; MMP2; IGF2; TIMP3; CDC42; TFAP4; RRAS2; ITGB1; WNT10B; TGFB2; CTNNB1; COL21A1; FGFR1; PLAU; HSPB2; FGF2; CD44; FLNC; FLNB; SOS1; RPS6; ITGB3; WNT7B; ERBB3
Rap1 signaling pathway	hsa04015	0.048226	PDGFRB; MLLT4; RAC2; KDR; RASSF5; PIK3R3; SIPA1L2; PDGFRA; PFN1; ADCY8; PLCB4; PLCG1; ADCY7; MAP2K6; TEK; NGF; NGFR; EPHA2; ITGB3; EFNA5; IGF1; EFNA3; CALM3; PDGFD; GNAI2; FGFR1; FGF2; ANGPT1; HGF; FGF10; CDH1; CDC42; GNAO1; ITGB1; CTNNB1; SIPA1L3; PLCB3; CSF1R; ACTB; FIGF; CSF1; ID1; PFN2; ANGPT4; THBS1; ADCY4; MAP2K3; VASP; FGF13; PARD3; CALM1; TLN2; FGF7; F2R; FGF5

DISCUSSION

In this study, we have identified several differentially expressed, novel lncRNA (TCONS_00046478, TCONS_00027225, and TCONS_00007697) that may play a role in osteogenic differentiation from iMSC#3by acting as miRNA (miR-689, miR-544 and miR-640) precursors and regulating the expression of co-expressed genes (COL4A4, COL21A1, and WNT2). These co-expressed genes are involved in cancer, ECM-receptor interaction, and focal adhesion pathways. miRNA participates in osteogenic differentiation from MSCs (Guo et al., 2011). Up-regulated miR-689 expression is involved in tumor necrosis factor-alpha (TNF-α)/RANKL-regulated osteoclast differentiation (Kagiya and Nakamura, 2013). miR-654-5p may play an important role in osteogenic differentiation from human bone marrow MSCsby directly suppressing themRNA and protein expressions of bone morphogenetic protein 2 (BMP2) by binding to a specific target site (Wei et al., 2012). The osteogenic differentiation function of TCONS_00046478 and TCONS_00027225, which also function as miR-689 and miR-654 precursors, may be mediated by miR-689 and miR-654 as the expression of miRNA and lncRNA appeared to be consistent (up-regulated during osteogenic differentiation). Although miR-640 is believed to be related to osteogenic differentiation in our study, no evidence of this has been documented so far. Therefore, there is a need to further study their roles in osteogenic differentiation.

Furthermore, we also assumed that lncRNA may play a role in osteogenic differentiation by regulating the cancer-related, ECM-receptor interaction, and focal adhesion pathways, by targeting the co-expressed genes (COL4A4, COL21A1, and WNT2). Several studies have reported that human MSCs can be induced to enter the osteogenic differentiation pathway in the presence of type I extracellular matrix (ECM) proteins (Salasznyk et al., 2004; Kihara et al., 2006) and type II collagen stimulates (Chiu et al., 2012). COL4A4 was also up-regulated during osteogenic differentiation from rat bone marrow stromal cells (Kaur et al., 2010). Type XXI collagen (COL21A1) is highly up-regulated in human MSCsin the presence of the peroxisome proliferator-activated receptor γ (PPARγ) inhibitor (GW9662), thereby enhancing osteoregeneration (Dudakovic et al., 2014). The Wnt signal transduction pathway is related to osteogenesis in human mesenchymal stem cells, via the canonical and non-canonical methods (Ling et al., 2009). Wnt3a, a representative canonical Wnt member, inhibits the osteogenic differentiation of mesenchymal stem cells, leads to reduced matrix mineralization, decreased alkaline phosphatase mRNA and activity, and lower osteoblastic marker gene expression (Boland et al., 2004; de Boeret al., 2004). On the other hand, the non-canonical Wnt members Wnt5a and Wnt4 promote osteogenesis (Boland et al., 2004; Chang et al., 2007). Wnt2 is also a canonical WNT ligand, and may also inhibit osteogenic differentiation. In line with the above theory, Wnt2 was downregulated in iMSCs, thereby promoting osteogenic differentiation in the DM group.

CONCLUSIONS

In conclusion, our findings have led to the identification of novel molecular mechanisms of osteogenic differentiation in MSCs from the perspective of lncRNA. Further studies regarding the target verification and function analysis of these lncRNA (TCONS_00046478, TCONS_00027225, and TCONS_00007697) could help provide conclusive evidence to explain lncRNA regulatory mechanism during the osteogenic differentiation of human MSCs.

Conflicts of interest

The authors declare no conflict of interest.

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