

Screening for genes, transcription factors and miRNAs associated with the myogenic and osteogenic differentiation of human adipose tissue-derived stem cells

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Abstract. In the present study, we aimed to reveal the molecular mechanisms responsible for the differentiation of human adipose tissue-derived stem cells (hASCs) into myocytes and osteoblasts. Microarray data GSE37329 were obtained from the Gene Expression Omnibus database, including three hASC cell lines from healthy donors, two osteogenic lineages and two myogenic lineages from the *in vitro*-induction of hASCs. Differentially expressed genes (DEGs) in the two lineages were firstly screened. Subsequently, the underlying functions of the two sets of DEGs were investigated by Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis, followed by protein-protein interaction (PPI) network construction. Regulatory relationships between transcription factors (TFs) and microRNAs (miRNAs or miRs) with target genes were finally explored using different algorithms. A total of 665 and 485 DEGs were identified from the hASC-derived myogenic and osteogenic lineages, respectively. The shared upregulated genes (n=205) in the two sets of DEGs were mainly involved in metabolism-related pathways, whereas the shared downregulated genes (n=128) were significantly enriched in the transforming growth factor- β (TGF- β) signaling pathway. Four genes, vascular endothelial growth factor A (*VEGFA*), fibroblast growth factor 2 (*FGF2*), nerve growth factor (*NGF*) and interleukin 1B (*IL1B*), presented with relatively higher degrees in both PPI networks. The transcription factor RAD21 was predicted to target shared upregulated and downregulated genes as well as specific downregulated genes in the myogenic and the osteogenic lineages. In addition, miRNA-DEG interaction analysis revealed that hsa-miR-1 regulated the most shared DEGs in the two lineages. There may

be a correlation between the four genes, *VEGFA*, *FGF2*, *IL1B* and *NGF*, and the differentiation of hASCs into myocytes and osteoblasts. The TF RAD21 and hsa-miR-1 may play important roles in regulating the expression of differentiation-associated genes.

Introduction

Human adipose tissue-derived stem cells (hASCs) are an attractive cell type for tissue engineering which may be harvested by direct excision or liposuction from human adipose tissue. Physiologically, hASCs are capable of differentiating into various lineages, such as adipocytes, osteoblasts, myocytes and chondrocytes (1,2). The ability of hASCs to undergo multilineage differentiation has attracted increasing interest in their use clinically and in regenerative medicine (3). A number of studies have suggested that hASCs possess significant potential for tissue rescue in multiple animal models, including heart failure, myocardial infarction, bone formation and wound healing, by differentiating into a variety of lineages (4-6).

Many factors have been reported to be involved in the mechanisms of hASC differentiation. Nutritional and hormonal signaling affects hASC differentiation in a negative or a positive manner, and the molecules involved in cell-matrix or cell-cell interactions play key roles in regulating the differentiation process (7-9). It is well known that fibroblast growth factor 2 (*FGF2*) inhibits the osteogenic differentiation of hASCs whereas it promotes chondrogenesis (10,11). Moreover, microRNA (miRNA or miR)-26a has been shown to modulate the late stage of osteoblast differentiation by targeting the transcription factor (TF) SMAD family member 1 (SMAD1) (4). The upregulation of miRNA-22 has been proved to promote the osteogenic differentiation of human adipose tissue-derived mesenchymal stem cells by suppressing histone deacetylase 6 (*HDAC6*) expression (12). Furthermore, hASCs are capable of differentiating into skeletal myocytes and cardiomyocytes under specific conditions (incubation in myogenic medium) (13,14). *In vitro*, sphingosylphosphorylcholine and transforming growth factor- β (TGF- β) induced the expression of smooth muscle-associated markers including α -smooth muscle actin, calponin and SM22 in hASCs (15,16). Numerous

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studies have been performed to reveal the molecular mechanisms controlling the differentiation of hASCs (7-16). However, the mechanisms responsible for the regulation of myocyte and osteocyte differentiation remain largely unknown.

Increasing evidence has proved that the conversion of hASCs into differentiated myocytes and osteocytes involves changes in gene expression which are mainly regulated by miRNAs and TFs (17,18). For instance, Luzi *et al* (4) showed that miR-26a expression was increased during hASC differentiation, whereas the expression of SMAD1 was complementary to that of miR-26a. In addition, Kim *et al* (17) reported that miR-196a regulates the differentiation and proliferation of hASCs by modulating the levels of the HOXC8 transcription factor.

To gain further insight into the molecular mechanisms responsible for the differentiation of hASCs into myocytes and osteocytes, we re-analyzed the microarray data GSE37329 through the identification of differentially expressed genes (DEGs) in hASC-derived myocytes and osteocytes compared with hASCs, as well as through functional annotation and protein-protein interaction (PPI) network construction. Furthermore, TFs and miRNAs targeting the DEGs were predicted and functionally analyzed.

Materials and methods

Gene datasets. The gene expression profile of GSE37329 was retrieved from the Gene Expression Omnibus (GEO) database available at <http://www.ncbi.nlm.nih.gov/geo/> (19). This dataset was deposited by Berdasco *et al* (19) on October 3, 2013 and was based on GPL11532 platform (Affymetrix Human Gene 1.1 ST array, Santa Clara, CA, USA). A total of 7 samples were available for further study, including three hASC cell lines from healthy donors, two osteogenic lineages and two myogenic lineages which were all obtained through the *in vitro* induction of hASCs.

Data preprocessing. The raw expression data (Affymetrix CEL files) were firstly preprocessed by the Robust Multiarray Average (RMA) normalization approach of Bioconductor affy package in R (20) (<http://www.bioconductor.org>), which returned the expression signals of each probe as log 2 scale. When different probes were mapped to the same gene, the mean value of the probes was considered as the gene value. Subsequently, the probe serial numbers in the matrix were transformed into gene names using the platform R/Bioconductor note package of the dataset chip. The matrix consisting of 20,253 genes was finally acquired.

Screening of DEGs. To screen out the DEGs in the *in vitro*-obtained osteogenic and myogenic lineages derived from hASCs compared with the freshly isolated hASCs obtained from healthy donors, respectively, Linear Models for Microarray Data (Limma) package of Bioconductor (21) was applied in the comparisons (osteogenic lineages vs. hASCs and myogenic lineages vs. hASCs). Unadjusted P-values were calculated using the Student's t-test. Genes with $P < 0.05$ and log 2IFC (fold change) ≥ 1 were considered to be differentially expressed. Hierarchical cluster analysis with the eligible DEGs was then performed in order to identify clusters of samples and genes.

Functional annotation of the DEGs. Functional enrichment of the two sets of DEGs in the osteogenic and the myogenic lineages *in vitro*-induced from the hASCs was assessed based on the biological process (BP) category in Gene Ontology (GO) (22) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation terms (23). GO and KEGG signaling pathway analyses were performed using the GO Function package (version 1.14.0) in Bioconductor (<http://www.bioconductor.org/packages/release/bioc/html/GOFunction.html>) (24), which conducted the standard hypergeometric test. A P-value < 0.05 was considered to indicate a statistically significant difference.

PPI network construction. Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org/>) is an online database which is comprised of more than 1,100 completely sequenced organisms and includes experimental as well as predicted interaction information (25). The up- and down-regulated genes in both sets of DEGs verified above were directly mapped to the STRING database in order to acquire significant PPI pairs which were previously verified by experiments, text mining and/or co-expressed analysis, respectively. Notable PPI pairs in which both of the genes were differentially expressed and the medium confidence was ≥ 0.4 were integrated to construct a PPI network. The network was visualized using CytoScape (26), available at <http://www.cytoscape.org>. Considering the complexity of PPI networks, we computed the degree of each node by measuring the numbers of links of the node in the network.

Computational identification of TFs. To determine the common mechanism responsible for the differentiation of hASCs into myocytes and osteocytes, DEGs shared in the osteogenic and the myogenic lineages were screened out. KEGG pathway enrichment analysis of the shared up- and downregulated genes was performed, respectively. P-values were calculated using hypergeometric distribution and a P-value < 0.05 was considered to indicate a significant pathway.

To further explore the molecular mechanism, eukaryotic TFs for the shared and unshared DEGs in osteogenic and myogenic lineages were collected based on the the Encyclopedia of DNA Elements (ENCODE) data from the UCSC Genome Browser (27) available at <http://genome.ucsc.edu/>. P-values were calculated using Fisher's exact test and adjusted using the Benjamini and Hochberg method to define the false discovery rate (FDR). Only the results with an FDR $< 5.5 \times 10^{-6}$ were considered to be significant.

miRNAs-target gene interaction network construction. To better understand the function of miRNAs in regulating the differentiation of hASCs, miRNAs targeting the shared up- and downregulated DEGs screened above were predicted using the miRecords database (28) available at <http://cl.accurascience.com/miRecords/> and the miRWalk database (29) available at <http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>. The miRNA-target interactions that were presented in miRecords and/or miRWalk and verified by experiment were used for the construction of the miRNA-mRNA interaction network. The network was visualized using CytoScape and the degree of each miRNA node was also measured. Furthermore, the predicted miRNAs were annotated with BP terms in the GO database.

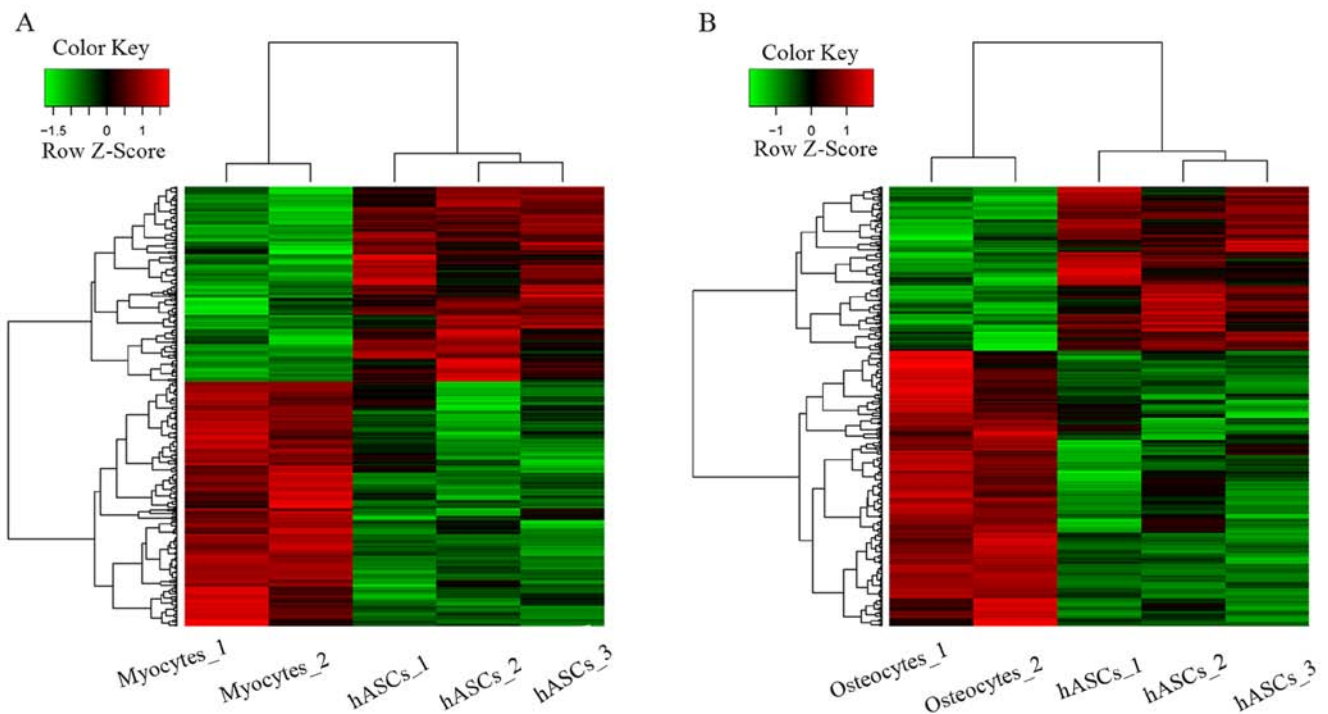


Figure 1. Hierarchical clustering of significantly differentially expressed genes in myocytes and osteocytes differentiated from human adipose tissue-derived stem cells (hASCs). (A) Clustering of genes in myocytes. (B) Clustering of genes in osteocytes. Red represents high expression, and the deeper the red color, a higher expression value. Green represents low expression, and a deeper green color, a lower the expression value.

P-values were calculated using hypergeometric distribution and GO terms with a P-value <0.05 were defined as significantly enriched.

Results

Screening of DEGs. Compared with the hASCs, 665 DEGs in myogenic lineages (370 up- and 295 downregulated genes) and 485 DEGs in osteogenic lineages (304 up- and 181 downregulated genes) were finally identified. The two sets of eligible DEGs were evaluated using unsupervised hierarchical clustering. As shown in Fig. 1, DEGs were found in different samples.

Annotating the biological functions of DEGs. To elucidate the functions of DEGs, the up- and downregulated genes in the *in vitro*-obtained myogenic and osteogenic lineages were mapped to BP terms in the GO database, and the top 10 GO terms are shown in Tables I and II, respectively. Briefly, the upregulated genes identified from the myogenic lineages were mainly involved in the regulation of multicellular organismal processes, inflammatory responses and cellular responses to chemical stimuli, whereas the downregulated genes were mainly involved in the regulation of multicellular organismal processes, single-multicellular organism processes, single-organism developmental processes and multicellular organismal development. On the other hand, the upregulated genes in the osteogenic lineages were mainly associated with responses to stimuli, regulation of multicellular organismal processes and regulation of localization, whereas the downregulated genes were mainly associated with anatomical structure development, system development and tissue development.

KEGG pathway enrichment analysis was used to further understand the biological functions of the DEGs. Analysis of the myogenic lineages revealed that the upregulated genes mainly participated in neuroactive ligand-receptor interactions and drug metabolism-cytochrome P450 pathways (Table III), which was the same as the upregulated genes in the osteogenic lineages (Table IV). By contrast, the downregulated genes in the myogenic lineages were mainly enriched in pathways in cancer, ECM-receptor interactions and focal adhesion (Table III), while the downregulated genes in the osteogenic lineages were mainly involved in the TGF- β signaling pathway and pathways in cancer (Table IV).

PPI network construction. There were 363 nodes and 996 edges in the PPI network of DEGs in myogenic lineages (Fig. 2). Based on the number of links, the top 8 nodes were identified as vascular endothelial growth factor A (VEGFA; degree, 57), interleukin (IL)6 (degree, 49), FBJ murine osteosarcoma viral oncogene homolog (FOS; degree, 41), FGF2 (degree, 37), jun proto-oncogene (JUN; degree, 35), IL1B (degree, 34), phosphoinositide-3-kinase, regulatory subunit 1 (PIK3R1; degree, 28) and nerve growth factor (NGF; degree, 27). In addition, 246 nodes and 520 edges constructed the PPI network of DEGs in the osteogenic lineages (Fig. 3), and the top 8 nodes were VEGFA (degree, 40), endothelin 1 (EDN1; degree, 24), IL1B (degree, 24), FGF2 (degree, 22), insulin-like growth factor 1 (IGF1; degree, 21), leptin (LEP; degree, 19), NGF (degree, 18) and matrix Gla protein (MGP; degree, 14). Considering the higher degree of VEGFA, IL1B, FGF2 and NGF in both networks, we hypothesized that these four genes play similar roles in the differentiation of hASCs into the two cell types.

Table I. Top 10 enriched GO terms in the BP category for both upregulated and downregulated differentially expressed genes in myocytes.

		GO ID	Name of BP	Count	P-value
Up	BP	GO:0051239	Regulation of multicellular organismal process	86	2.28E-11
		GO:0006954	Inflammatory response	35	2.20E-09
		GO:0070887	Cellular response to chemical stimulus	83	2.25E-09
		GO:0042221	Response to chemical	115	2.87E-08
		GO:0050896	Response to stimulus	198	6.91E-08
		GO:0032879	Regulation of localization	70	7.58E-08
		GO:0050727	Regulation of inflammatory response	20	7.83E-08
		GO:0006805	Xenobiotic metabolic process	16	7.94E-08
		GO:0050793	Regulation of developmental process	67	8.64E-08
		GO:0071466	Cellular response to xenobiotic stimulus	16	8.68E-08
Down	BP	GO:0001944	Vasculature development	43	0
		GO:0007275	Multicellular organismal development	147	0
		GO:0009653	Anatomical structure morphogenesis	104	0
		GO:0009888	Tissue development	75	0
		GO:0030154	Cell differentiation	118	0
		GO:0032501	Multicellular organismal process	173	0
		GO:0032502	Developmental process	156	0
		GO:0044707	Single-multicellular organism process	172	0
		GO:0044767	Single-organism developmental process	153	0
		GO:0048731	System development	139	0

Up, upregulated; down, downregulated; GO, gene ontology; BP, biological process.

Table II. Top 10 enriched GO terms in the BP category for both upregulated and downregulated differentially expressed genes in osteocytes.

		GO ID	Name of BP	Count	P-value
Up	BP	GO:0050896	Response to stimulus	174	2.59E-10
		GO:0006805	Xenobiotic metabolic process	15	3.63E-08
		GO:0071466	Cellular response to xenobiotic stimulus	15	3.96E-08
		GO:0032879	Regulation of localization	61	5.48E-08
		GO:0009410	Response to xenobiotic stimulus	15	6.02E-08
		GO:0051239	Regulation of multicellular organismal process	64	4.22E-07
		GO:0051049	Regulation of transport	48	4.74E-07
		GO:0006954	Inflammatory response	27	5.39E-07
		GO:0051046	Regulation of secretion	26	7.18E-07
		GO:1901700	Response to oxygen-containing compound	42	1.88E-06
Down	BP	GO:0072358	Cardiovascular system development	33	4.90E-12
		GO:0072359	Circulatory system development	33	4.90E-12
		GO:0014706	Striated muscle tissue development	20	3.27E-11
		GO:0060537	Muscle tissue development	20	6.71E-11
		GO:0048731	System development	74	1.71E-10
		GO:0001944	Vasculature development	24	9.03E-10
		GO:0048856	Anatomical structure development	80	1.07E-09
		GO:0009888	Tissue development	42	3.06E-09
		GO:2000026	Regulation of multicellular organismal development	37	3.14E-09
		GO:0009653	Anatomical structure morphogenesis	51	4.97E-09

Up, upregulated; down, downregulated; GO, gene ontology; BP, biological process.

Table III. Top 10 enriched KEGG pathways of upregulated and downregulated differentially expressed genes in myocytes.

	KEGG ID	Name	Count	P-value
Up	00982	Drug metabolism - cytochrome P450	9	2.36E-05
	00350	Tyrosine metabolism	5	0.001759753
	05145	Toxoplasmosis	8	0.007544112
	00460	Cyanoamino acid metabolism	2	0.009086159
	00071	Fatty acid metabolism	4	0.013452544
	05014	Amyotrophic lateral sclerosis (ALS)	4	0.027079145
	04080	Neuroactive ligand-receptor interaction	11	0.032800823
	00603	Glycosphingolipid biosynthesis - globo series	2	0.035670336
	00590	Arachidonic acid metabolism	4	0.038155628
	00120	Primary bile acid biosynthesis	2	0.045739216
Down	04512	ECM-receptor interaction	12	5.83E-08
	04350	TGF- β signaling pathway	11	4.72E-07
	05323	Rheumatoid arthritis	10	8.16E-06
	04510	Focal adhesion	14	2.53E-05
	04640	Hematopoietic cell lineage	9	4.23E-05
	05200	Pathways in cancer	18	4.23E-05
	04514	Cell adhesion molecules (CAMs)	10	0.000217686
	05144	Malaria	6	0.000396575
	05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	7	0.000505663
	05217	Basal cell carcinoma	6	0.000599721

ECM, extracellular matrix; TGF- β , transforming growth factor- β ; up, upregulated; down, downregulated; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table IV. Top 10 enriched KEGG pathways of upregulated and downregulated differentially expressed genes in osteocytes.

	KEGG ID	Name	Count	P-value
Up	00982	Drug metabolism - cytochrome P450	10	5.30E-07
	00350	Tyrosine metabolism	6	7.68E-05
	04080	Neuroactive ligand-receptor interaction	14	0.000309764
	04270	Vascular smooth muscle contraction	8	0.001033472
	00460	Cyanoamino acid metabolism	2	0.006279036
	00071	Fatty acid metabolism	4	0.006987698
	00260	Glycine, serine and threonine metabolism	3	0.018947162
	00590	Arachidonic acid metabolism	4	0.020746069
	00603	Glycosphingolipid biosynthesis - globo series	2	0.025079512
	00010	Glycolysis/gluconeogenesis	4	0.028465778
Down	04350	TGF- β signaling pathway	7	2.00E-05
	04610	Complement and coagulation cascades	4	0.005201809
	05217	Basal cell carcinoma	3	0.018290654
	04916	Melanogenesis	4	0.01930188
	04972	Pancreatic secretion	4	0.01930188
	04710	Circadian rhythm - mammal	2	0.020821702
	00512	Mucin type O-Glycan biosynthesis	2	0.037222845
	04360	Axon guidance	4	0.042211972
	05200	Pathways in cancer	7	0.04712619
	05020	Prion diseases	2	0.049293741

TGF- β , transforming growth factor- β ; up, upregulated; down, downregulated; KEGG, Kyoto Encyclopedia of Genes and Genomes.

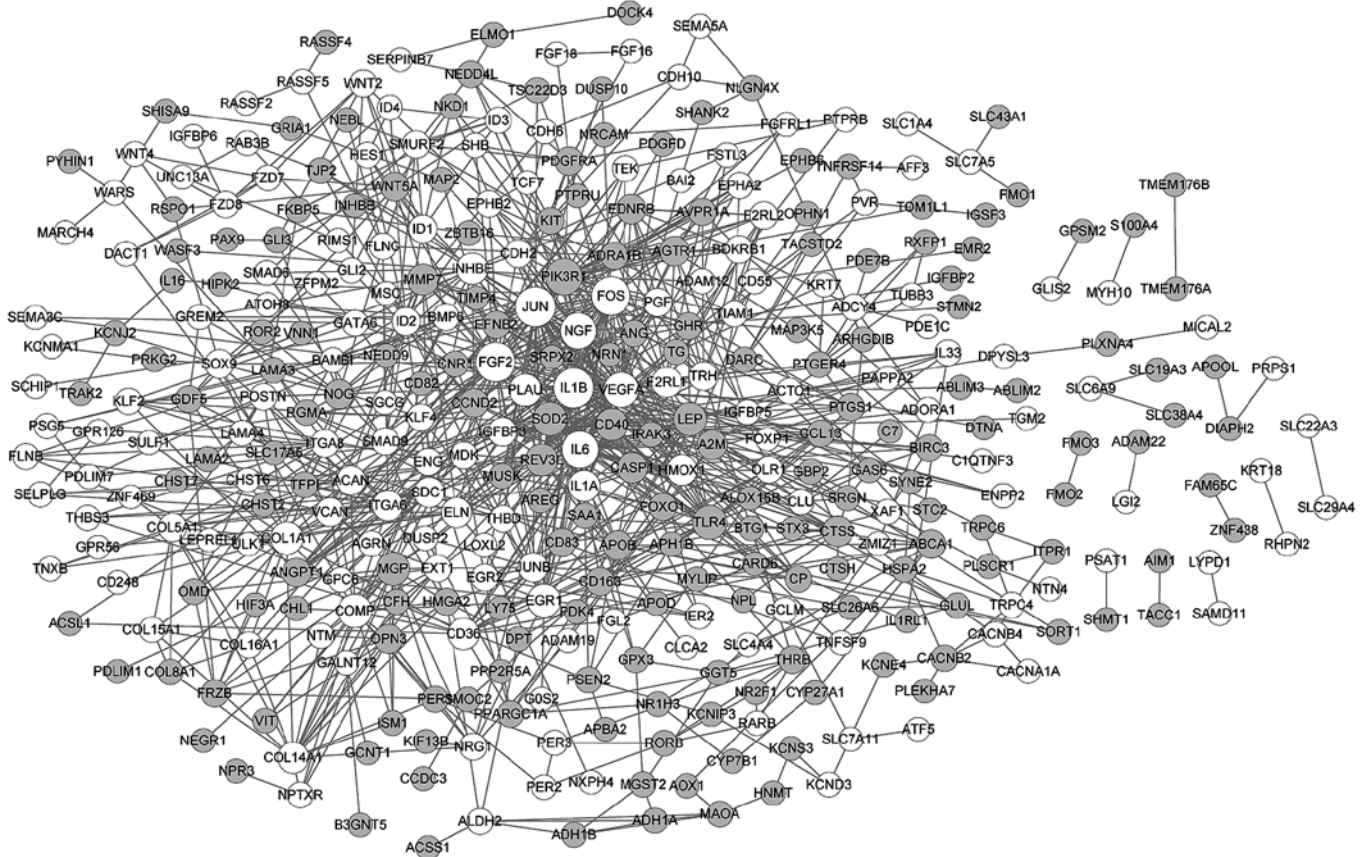


Figure 2. Protein-protein interaction network of differentially expressed genes in myocytes. Gray nodes represent downregulated genes and white nodes represent upregulated genes in myocytes. The higher the degree, the larger the node size.

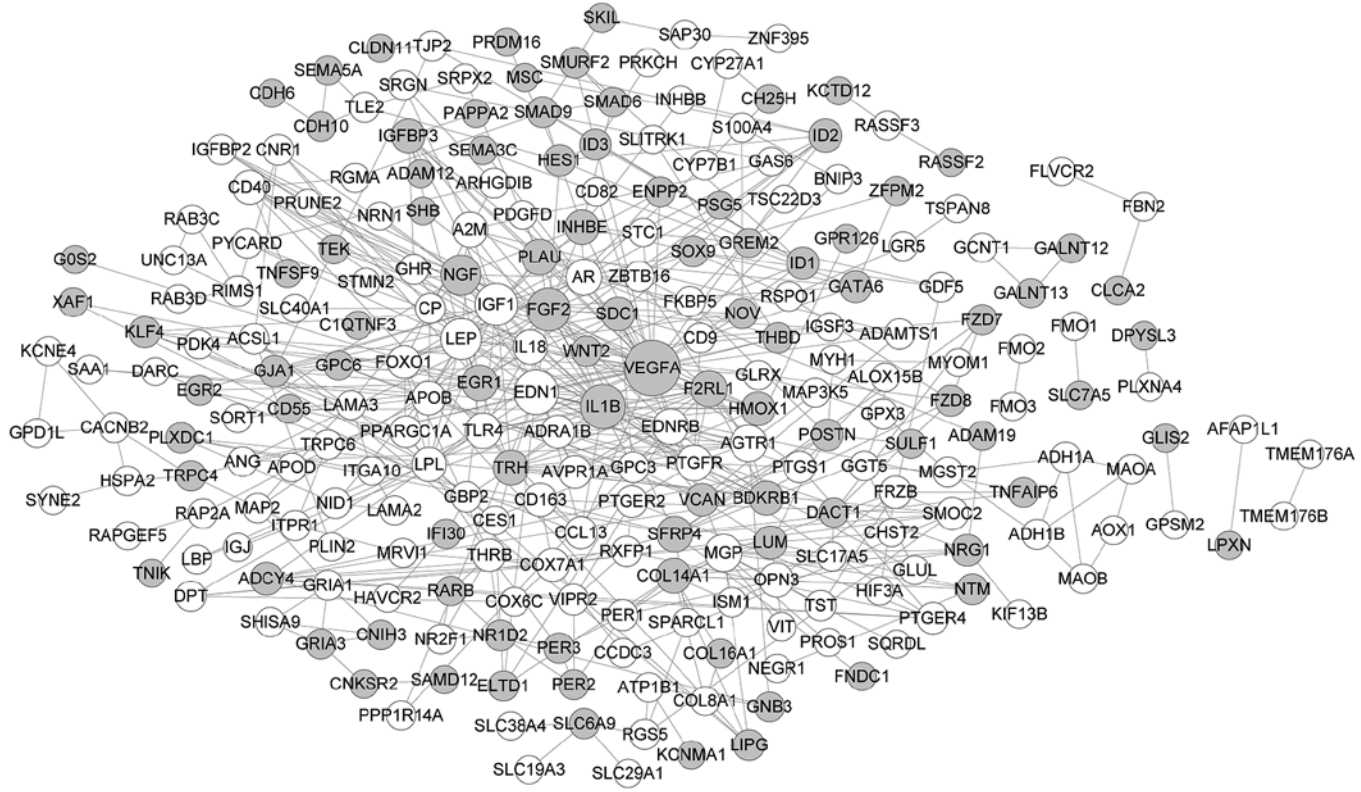


Figure 3. Protein-protein interaction network of differentially expressed genes in osteocytes. Gray nodes represent downregulated genes and white nodes represent upregulated genes in osteocytes. The higher the degree, the larger the node size.

Table V. Enriched KEGG pathways of shared genes between two groups (myocytes vs. hASCs and osteocytes vs. hASCs).

	KEGG ID	Name of pathway	Count	P-value
Shared up	00982	Drug metabolism - cytochrome P450	9	2.80E-07
	00350	Tyrosine metabolism	5	0.000155935
	04080	Neuroactive ligand-receptor interaction	11	0.000601223
	00071	Fatty acid metabolism	4	0.002082258
	00460	Cyanoamino acid metabolism	2	0.003246415
Shared down	04350	TGF- β signaling pathway	7	3.78E-06
	04610	Complement and coagulation cascades	4	0.002133141
	04916	Melanogenesis	4	0.008361484
	04972	Pancreatic secretion	4	0.008361484

TGF- β , transforming growth factor- β ; up, upregulated; down, downregulated; KEGG, Kyoto Encyclopedia of Genes and Genomes; hASCs, human adipose-derived stem cells.

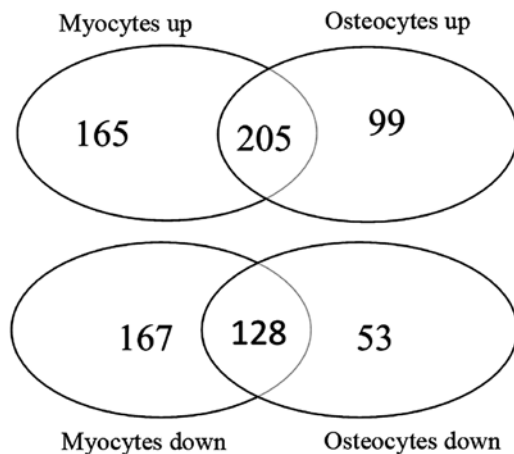


Figure 4. Venn diagram of shared genes and unshared genes identified by comparisons (myocytes vs. human adipose tissue-derived stem cells (hASCs) and osteocytes vs. hASCs). The overlapping genes are shared genes. The number of shared upregulated (up) and shared downregulated (down) genes is shown.

Enrichment analysis of TFs. To further explore the molecular mechanisms responsible for the differentiation of hASCs into myocytes and osteocytes, the shared and unshared DEGs in the *in vitro*-obtained osteogenic and myogenic lineages were analyzed, respectively (Fig. 4). The results of the KEGG enrichment analysis revealed that 205 shared upregulated genes were mainly involved in metabolism-related pathways, including drug metabolism and tyrosine metabolism, and 128 shared downregulated genes were significantly enriched in the TGF- β signaling pathway (Fig. 4 and Table V).

The relationship between TFs and DEGs may aid in defining regulatory controls. Finally, a total of 27 TFs targeting the shared upregulated genes were predicted. In addition, 11 TFs, which are all involved in the targeting of the shared upregulated genes, were predicted to target the shared downregulated genes, including RAD21, zinc finger protein 263 (ZNF263), signal transducer and activator of transcription 3 (STAT3), RE1-silencing transcription factor (REST, also known as NRSF), tripartite motif containing 28 (TRIM28, also known as KAP1), GATA binding protein 2 (GATA2), CCCTC-binding

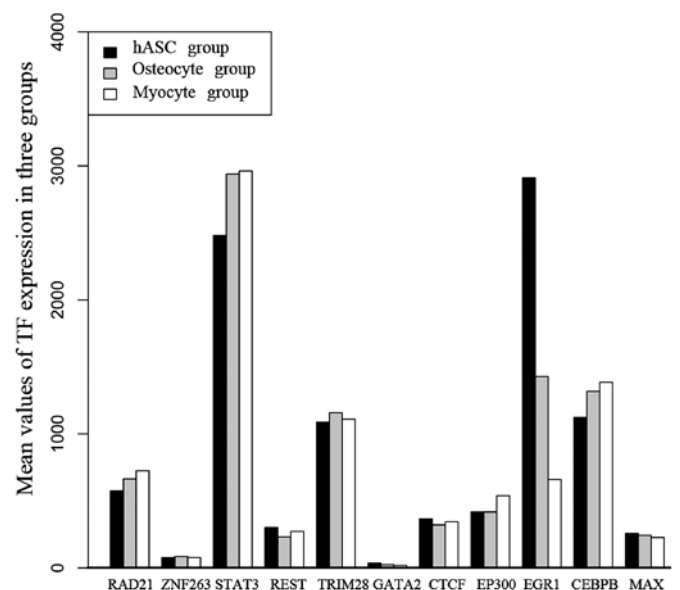


Figure 5. The mean expression of 11 transcription factors (TFs) in three cell lineages including human adipose tissue-derived stem cells (hASCs), myocytes and osteocytes. The horizontal axis represents TFs and the vertical axis represents the mean expression value of the TFs.

factor (CTCF), E1A binding protein p300 (EP300), early growth response 1 (EGR1), CCAAT/enhancer binding protein (C/EBP), beta (CEBPB) and MYC-associated factor X (MAX). The expression of these 11 TFs in the three sample types is shown in Fig. 5. The results revealed that the expression of EGR1 was significantly higher in the hASCs than in the osteogenic and the myogenic lineages. Conversely, the expression of STAT3 was significantly lower in the hASCs than in the osteogenic and the myogenic lineages. Differential expression of the other 9 TFs among the three cell types was not found.

In addition, 26 and 21 TFs were predicted to regulate the unshared up- and downregulated genes in the myogenic lineages, respectively. In the osteogenic lineages, 11 TFs were predicted to target the upregulated genes whereas only RAD21 was found to regulate the downregulated

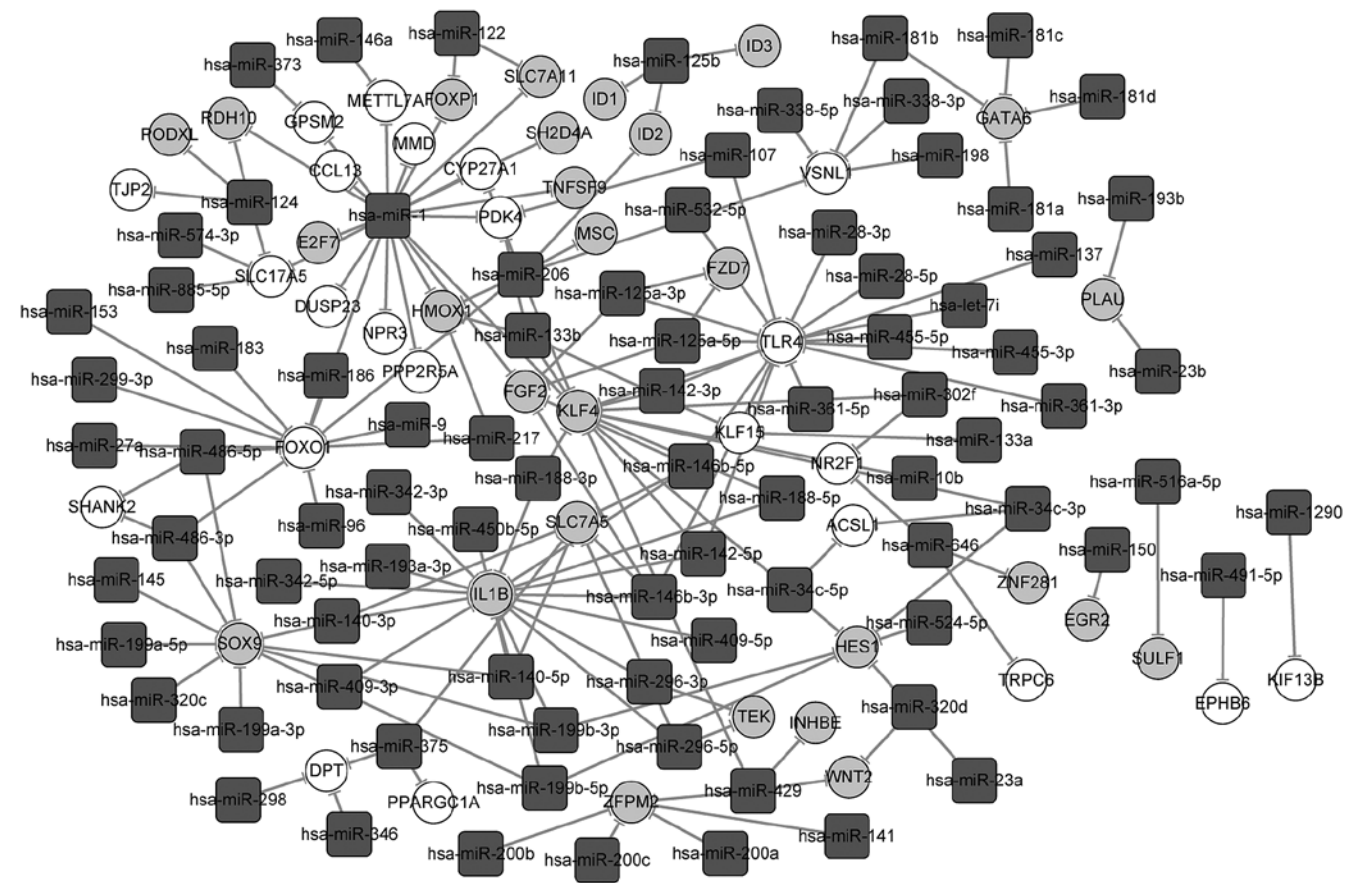


Figure 6. miRNA-target gene interaction network. The square nodes represent miRNA and the circle nodes represents the target gene. White nodes represent upregulated genes in myocytes and osteocytes. Light gray nodes represent downregulated genes in myocytes and osteocytes.

genes. Moreover, RAD21 was also included among the TFs regulating unshared downregulated genes in the myogenic lineages, including *VEGFA* and SMAD family member 6 (*SMAD6*).

MiRNA-DEG interaction analysis. A total of 66 and 98 miRNA-mRNA pairs were finally screened out for the shared up- and downregulated genes in the osteogenic and the myogenic lineages to construct an miRNA-target gene interaction network, respectively (Fig. 6). In the network, hsa-miR-1, with the highest degree, regulated 20 common genes differentially expressed in the two cell types, including Forkhead box P1 (*FOXPI*), E2F transcription factor 7 (*E2F7*), chemokine (C-C motif) ligand 13 (*CCL13*), monocyte to macrophage differentiation-associated (*MMD*) and pyruvate dehydrogenase kinase, isozyme 4 (*PDK4*). Moreover, the shared upregulated genes *FOXO1*, *TLR4* and downregulated gene *IL1B* were regulated by >9 miRNAs during the differentiation of hASCs, and shared downregulated *GATA6* was regulated by four hsa-miR-181 family members namely miR-181a, miR-181b, miR-181c and miR-181d.

Further, functional annotation revealed that the shared upregulated genes targeted by the predicted miRNAs were mainly involved in immune response-related BPs, including detection of fungus, and host defense responses. By contrast, the shared downregulated genes were significantly enriched in response to ozone, smooth muscle adaptation and regulation of myosin light chain kinase activity (Table VI).

Discussion

In the present study, we aimed to extend our understanding of the molecular mechanisms responsible for the differentiation of hASCs into myocytes and osteocytes. We found that four proteins encoded by *VEGFA*, *FGF2*, *NGF* and *IL1B* were differentially expressed in the myogenic and the osteogenic lineages and presented in the PPI network at relatively high degrees. Moreover, the TF RAD21 was predicted to target both shared up- and downregulated genes as well as specific downregulated genes in the myogenic and the osteogenic lineages. In addition, miRNA-DEG interaction analysis revealed that hsa-miR-1 regulated the most shared DEGs in the two lineages, such as *FOXPI* and *CCL13*.

Previous findings have suggested that hASCs secrete significant numbers of angiogenic factors, including *VEGFA* (30). *VEGFA* is known to promote both angiogenesis and osteogenesis (31,32). More recently, *VEGFA* has been proved to play an integral role in the crosstalk between endothelial cells and osteoblasts and is also considered as being of great importance for vascularization (33). *VEGFA* has been found to increase bone formation, promote osteoblast differentiation and inhibit the apoptosis of osteoblasts (32,34). In addition, Song *et al* have identified *VEGF* as a critical factor in cardiomyogenesis in hASCs (35). *FGF2*, a member of the FGF family, has been identified as a major candidates for the regulation of self-renewal in human embryonic stem cells (36,37). *FGF2* may also be important in increasing the lifespan of bone marrow stromal cells and

Table VI. Top 7 enriched GO terms in the BP category for target genes of miRNAs.

		GO ID	Name of BP	Count	P-value
miRNA-gene-up	BP	GO:0016046	Detection of fungus	16	3.05E-14
		GO:0052031	Modulation by symbiont of host defense response	16	8.69E-14
		GO:0052033	Pathogen-associated molecular pattern dependent induction by symbiont of host innate immune response	16	8.69E-14
		GO:0052166	Positive regulation by symbiont of host innate immune response	16	8.69E-14
		GO:0052167	Modulation by symbiont of host innate immune response	16	8.69E-14
		GO:0052169	Pathogen-associated molecular pattern dependent modulation by symbiont of host innate immune response	16	8.69E-14
		GO:0052255	Modulation by organism of defense response of other organism involved in symbiotic interaction	16	8.69E-14
miRNA-gene-down	BP	GO:0010193	Response to ozone	17	0
		GO:0014805	Smooth muscle adaptation	21	0
		GO:0035504	Regulation of myosin light chain kinase activity	17	0
		GO:0035505	Positive regulation of myosin light chain kinase activity	17	0
		GO:0060352	Cell adhesion molecule production	17	0
		GO:0060353	Regulation of cell adhesion molecule production	17	0
		GO:0060355	Positive regulation of cell adhesion molecule production	17	0

Up, upregulated; down, downregulated; GO, Gene Ontology; BP, biological process;

for supporting proliferation as well as the chondrogenic and osteogenic differentiation potential (38,39). Moreover, previous studies have shown that the exposure of hASCs to FGF2 led to the enhancement of chondrogenic lineage differentiation and the inhibition of osteogenic lineage differentiation, as well as the stimulation of adipogenic differentiation (10,40,41). Notably, IL1B, which encodes an inflammatory cytokine, has been shown to be suppressed by mesenchymal stem cell (MSC) transplantation at the transcriptional and the post-transcriptional levels in myocardial infarction (42). NGF is also reported to be associated with many pathologic and physiologic processes, such as differentiation of stem cells (43). In this study, VEGFA, FGF2, IL1B and NGF were found to be downregulated in the myogenic and osteogenic lineages compared with hASCs and connected with relatively more DEGs in the PPI networks, which supports the hypothesis that there may be a correlation between these genes and the differentiation of hASCs.

Additionally, TFs and miRNAs are essential regulatory molecules after DNA replication involved in the differentiation of hASCs. The TF RAD21 has been proved to be associated with the maintenance of embryonic stem cell identity through association with the pluripotency transcriptional network (44). Consistent with our analysis, chromatin immunoprecipitation analysis was used in a previous study to confirm that VEGFA and SMAD6 expression is regulated by RAD21 (45). SMAD6, an inhibitory SMAD, has been reported to inhibit the TGF- β signaling pathway that suppresses osteoblast and myogenic differentiation (46). The data from the present study revealed that RAD21 mediates the differentiation of hASCs by regulating the expression of VEGFA and SMAD6.

In a previous study, miR-1 was shown to strongly enhance myogenesis following the transfection of myoblasts with hsa-miR-1 by modulating skeletal muscle proliferation and

differentiation (47). More importantly, hsa-miR-1 is required for smooth muscle cell lineage differentiation from embryonic stem cells by binding with the 3' untranslated region of the gene encoding Kruppel-like factor 4 (48). Following the construction of an miRNA-target gene interaction network, we found that miR-1 targeted FOXP1 in the differentiation of hASCs into osteocytes and myocytes, which is in agreement with the results of a previous study (49). Additionally, it was demonstrated that knockdown of FOXP1 suppressed the self-renewal capacity of MSCs and reduced the osteogenic potential (50). In the hASC-derived myocytes and osteocytes, CCL13 was upregulated which is consistent with the findings of a previous study revealing a 12-fold change after culturing hASCs with proinflammatory cytokines (51). Our results suggest that miR-1 modulates the differentiation of hASCs into myocytes and osteocytes by regulating FOXP1 and CCL13.

In conclusion, we performed a comprehensive bioinformatics analysis of the expression profiles of *in vitro*-induced osteogenic and myogenic lineages and hASC cell lines from healthy donors. There may be a correlation between four shared downregulated genes in the two lineages, VEGFA, FGF2, IL1B and NGF, and the differentiation of hASCs. Notably, the TF RAD21 and hsa-miR-1 may play important roles in regulating the expression of differentiation-associated genes. This study may provide new insight into the underlying molecular mechanisms of hASC differentiation, which may help to repair and reconstruct damaged organs. However, further studies are warranted to confirm these results and to clarify their roles in the differentiation of hASCs.

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