Iran. J. Biotechnol. January 2021;19(1): e2565 DOI: 10.30498/IJB.2021.2565
Research Article

Topological Analysis of Regulatory Networks Reveals Functionally Key Genes and miRNAs Involved in the Differentiation of Mesenchymal Stem Cells

Atousa Ataei[1t], Mansour Poorebrahim[2t], Azam Rajabpour[3], Albert Rizvanov[4], Seyed Shahriar Arab[5*]

[1] Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia.
[2] Department of Medical Biotechnology, School of Advanced Technologies in Medicine, University of Medical Sciences, Tehran, Iran.
[3] Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.
[4] Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia.
[5] Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
[t] Equal contribution

**Corresponding author:* Seyed Shahriar Arab. Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran. Tel: +98-2182883494, Fax: +98- 2182884717, E-mail: sh.arab@modares.ac.ir

Background: The details of molecular mechanisms underlying the differentiation of Mesenchymal Stem Cells (MSCs) into specific lineages are not well understood.
Objectives: We aimed to construct the interactome network and topology analysis of bone marrow mesenchymal stem cell of CAGE data. Applying the enrichment results, we wanted to introduce the common genes and hub-microRNA and hub-genes of these giant network.
Materials and Methods: In this study, we constructed gene regulatory networks for each non-mesenchymal cell lineage according to their gene expression profiles obtained from FANTOM5 database. The putative interactions of TF-gene and protein-protein were determined using TRED, STRING, HPRD and GeneMANIA servers. In parallel, a regulatory network including corresponding miRNAs and total differentially expressed genes (DEGs) was constructed for each cell lineage.
Results: The results indicated that analysis of networks' topology can significantly distinguish the hub regulatory genes and miRNAs involved in the differentiation of MSCs. The functional annotation of identified hub genes and miRNAs revealed that several signal transduction pathways i.e. AKT, WNT and TGFβ and cell proliferation related pathways play a pivotal role in the regulation of MSCs differentiation. We also classified cell lineages into two groups based on their predicted miRNA profiles.
Conclusions: In conclusion, we found a number of hub genes and miRNAs which seem to have key regulatory functions during differentiation of MSCs. Our results also introduce a number of new regulatory genes and miRNAs which can be considered as the new candidates for genetic manipulation of MSCs in vitro.

Key words: Differentiation, MSCs, miRNA, Regulatory network, Topological analysis

1. Background

Mesenchymal stem cells (MSCs) can be isolated in adults from a variety of tissues including: bone marrow, adipose tissue, liver, dental pulp, endometrium, muscle, amniotic fluid, placenta and umbilical cord blood. Because of their capacity for self-renewal and multipotency, MSCs are widely used in cell-based therapy for a wide range of autoimmune disorders (1), degenerative diseases (2) and cancers (3). MSCs have a high potential to differentiate into several distinct cell lineages namely osteoblasts, chondrocytes, adipocytes, muscle cells, cardiomyocytes, tenocyte and neural precursors. Furthermore, these cells actively participate in organ homeostasis, injury repair, and aging processes. However, the details of mechanisms underlying differentiation of MSCs are only in part understood and it must be considered that there are some outstanding ambiguities in the differentiation stages of MSCs. In order to provide new insights into the biological aspects of differentiation of MSCs, there has been a growing effort toward monitoring the distinct state of stem cells differentiation using high-

Copyright *© 2021 The Author(s); Published by National Institute of Genetic Engineering and Biotechnology. This is an open access article, distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses /by-nc/4.0/) which permits others to copy and redistribute material just in noncommercial usages, provided the original work is properly cited.*

throughput biochips (4). There are a few regulatory genes reported as a key regulator of osteogenesis (RUNX2, SP7), chondrogenesis (SOX9), adipogenesis (PPARG, C/EBPs), myogenesis (MYOD1, MYF5), tenogenesis (TNMD) and neurogenesis (SOX2, NEUROG2). Despite these findings, in vitro production of terminally differentiated cells from MSCs has been remained a challenge yet. Therefore, identification of important regulatory genes and miRNAs involved in the differentiation of MSCs seems to be critical for successful stem cell-based tissue engineering. Yang et al. characterized the essential genes using topological analysis of protein-protein interaction (PPI) networks and found that this strategy could accurately separate essential genes from non-essential genes in Saccharomyces cerevisiae (5). There are several studies that indicate topological analysis of PPI networks is highly capable of finding new biomarkers in various medical investigations (6). Most recently, we evaluated the role of Pluronic P85 treatment in transcription profile of human tooth germ stem cells (hTGSCs) by a network-based approach. Our results could efficiently clarify the molecular mechanisms underlying the impact of Pluronic P85 on hTGSCs differentiation (7).

MiRNAs are a group of short non-coding endogenous RNAs known to post-transcriptionally control gene expression and influence a broad range of biological processes. It has been previously demonstrated that the complex regulatory network of miRNAs-TFs could be associated with a number of common disorders (8). An interesting field of research has been generated to identify the family of miRNAs that are suspected to contribute in the differentiation of MSCs (9). Recently emerging evidences demonstrate that differentiation of MSCs is closely dependent on the miRNA expression profile regulating critical steps of MSCs differentiation into particular lineages (10, 11). Based on the low-throughput studies, some miRNAs have been reported as key modulators of MSCs gene expression during differentiation (12-15). However, each miRNA may regulate hundreds of genes and several miRNAs can impair the expression of a single functional gene. Taken together, it seems necessary to comprehensively investigate miRNAs-genes interactions in the MSCs during differentiation processes. It is also important to know that miRNA expression patterns induced during differentiation of MSCs can consider as a reliable signature of lineage determination (16). In particular, a set of multiple human differentiated tumors have been successfully clustered based on miRNA expression profiles, whereas messenger RNA (mRNA) profiles couldn't accurately classify the same samples (17).

2. Objectives

In the present study, we constructed TF-gene and protein-protein regulatory networks of six possible differentiation fates of MSCs including: osteogenesis, chondrogenesis, adipogenesis, myogenesis, neurogenesis and tenogenesis based on the experimental data obtained from cap analysis gene expression (CAGE). Subsequently, we merged these constructed networks together and built a final PPI network for each cell type. In parallel, a detailed miRNAs-PPI regulatory network also was reconstructed for each differentiation pathways. Topological analysis of the resulted regulatory networks revealed functionally distinct miRNAs and genes, which seemed to be involved in the differentiation of MSCs. Finally, we clustered cell lineages according to their predicted miRNA profiles.

3. Materials and Methods

3.1. Data Collection

In the present study gene expression data of several non-mesenchymal lineages including: osteocyte (3 samples), adipocyte (5 samples), myocyte (4 samples), chondrocyte (2 samples), tenocyte (3 samples), neurocyte (3 samples) and mesenchymal bone marrow stem cells (3 samples) were collected from Functional annotation of mammalian genomes5 (FANTOM5) database (18). The information regarding selected datasets is available in supplementary (**Table S1**). FANTOM5 encompasses transcription regulatory data across a variety of biological states in the mammalian cells. Normalization steps and subsequently RNA-Seq data analysis were performed using DESeq package (19) in R (https://www.r-project.org/). DESeq works based on negative binomial distribution and it has been shown that it outperforms other existing methods for finding DEGs, therefore, it can better fit to expressional data. Finally, the DEGs of six lineages were identified compared to bone marrow mesenchymal stem cell samples. We considered fold change (log2 fold changes larger than 1.5 and smaller than -1.5) and p-value below 0.05 as cutoffs to select the important genes from normalized data.

3.2. Regulatory Network Construction

Among detected DEGs in six understudied cell lineages and omitting the genes with no known function, the remained functional genes were selected for constructing gene regulatory networks. Protein-protein relationships' data were obtained from STRING (a protein-protein interaction database) (20), GeneMANIA (a web service used for prediction of gene function for a list of genes)

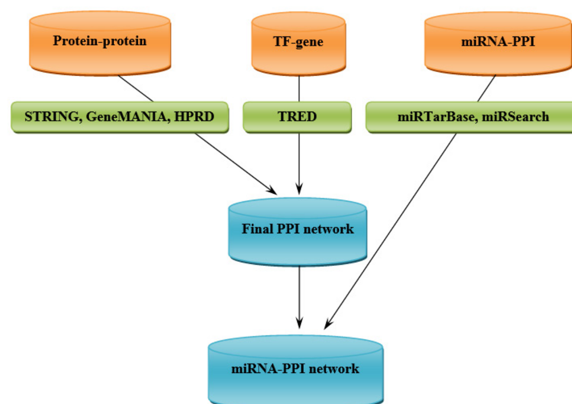

Figure 1. Schematic overview of the overall steps taken to construct regulatory networks of six corresponding cell lineages.

(21) and Human Protein Reference Database (HPRD; http://www.hprd.org/) databases as well as literature mining (**Fig. 1**). The putative TF-target gene pairs were obtained from TRED (The transcriptional regulatory element database) (22). Only the interactions with at least one published experimentally validation were retrieved from the databases. The corresponding protein-protein and TF-gene networks in all six cell lineages were constructed. TF-genes and protein-protein networks were merged together and a final PPI network was built for each cell lineage. Briefly, The TFs were initially filtered from DEG lists via utilizing TRED database, and subsequently a TF-gene regulatory network was constructed for DEGs of each cell lineage. In this step, only the gene targets that were existed in DEGs list participated in network construction. In parallel, PPIs were collected from STRING, HPRD and GeneMANIA. All found interactions were integrated into a file and visualized using Cytoscape for further analyses. In parallel, the experimentally validated miRNAs involved in the regulation and function of TFs and other genes in PPI networks were collected through text mining beside mirTarBase (23) and miRSearch (https://www.exiqon.com/mirsearch) databases. Only experimentally verified miRNA–target relationships were considered for further analyses. Corresponding miRNA-PPI networks in the respective cell types were also reconstructed.

3.3. Networks Topological Analysis

Topological network analysis was carried out for PPI and miRNA-PPI networks of all six cell lineages. Among different measures available for network topology analysis using Cytoscape 3.2.1 plugin Network Analyzer, Degree and Betweenness centrality were selected as a criterion for selecting important regulatory genes in this study, since their results showed better outcome than others (highly consistent with MSCs differentiation pathways) (24). Nodes degree which is one of the centrality measures of a network refers to the number of neighbors (edges) of a node. Usually nodes with higher degree centrality are considered as biologically important ones within biological networks. Betweenness centrality is another centrality measure representing the number of times each node is visited during traversing all shortest paths (the smallest number of edges linking any pair of nodes) and computed as follow:

$$C_b(n) = \sum_{s \neq n \neq t} (\sigma_{st}(n)/\sigma_{st})$$

The s and t are nodes in the network different from n, σ_{st} indicate the number of shortest paths from s to t, and $\sigma_{st}(n)$ is the number of shortest paths from s to t that n lies on.

We selected top genes according to the ranked results of two mentioned methods. The genes with high Betweenness centrality or degree and miRNAs with higher out-degree were considered as important regulatory hubs.

3.4. Functional Annotation Clustering

In order to determine the functional categories of the regulatory hub genes obtained from the previous step, the list of detected hub genes in each network were introduced to DAVID bioinformatics resources (25). Hub genes with transcriptional regulatory function were selected in order to identify any regulatory relationships among them. Interactions between transcriptional regulatory hub genes and corresponding hub miRNAs were determined and visualized (**Fig. 2**). Specific enrichment of miRNAs was also performed using TAM tool (26).

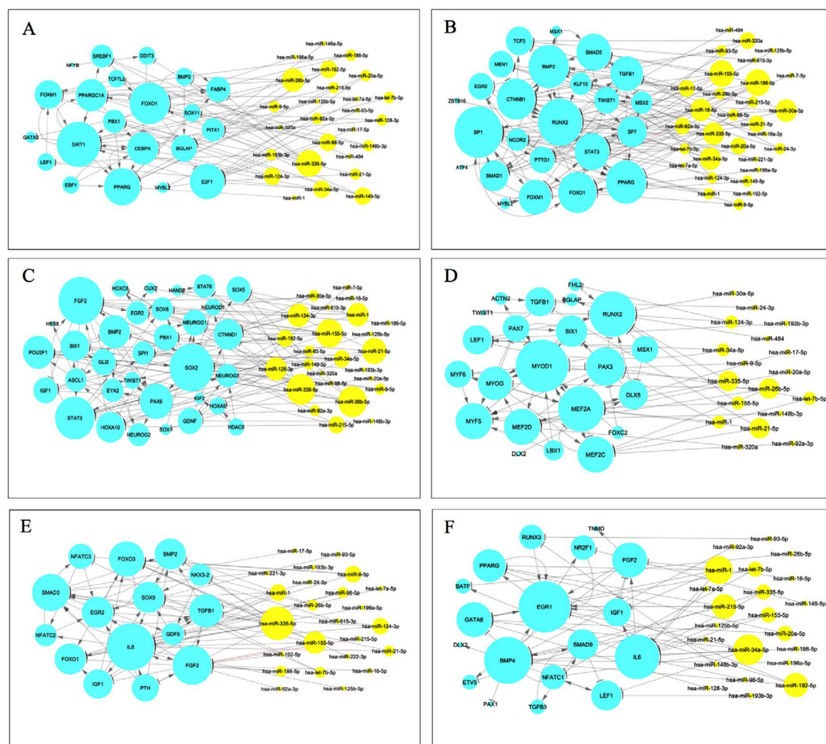

Figure 2. The representation of hub regulatory genes (Cyan) and miRNAs (Yellow) along with their interactions in the six lineages. Nodes with higher Degree are shown in the bigger circles. (A) Adipocyte, (B) Osteocyte, (C) Neurocyte, (D) Myocyte, (E) Chondrocyte, (F) Tenocyte.

3.5. MiRNA-Based Classification of Cell Lineages

The classification of cell lineages were performed in MATLAB 2014 based on respective miRNA profiles with a distance-based hierarchical clustering (using Jaccard similarity coefficient distance (27)) method written in JAVA programming language. Jaccard similarity coefficient is used for the comparison of the similarity of datasets through calculating the intersection of two sample sets divided by their unions. In this study first the Jaccard distance between 6 different cell lineages were calculated using a written program in JAVA. The resulted distance table was in turn imported to MATLAB 2014 and classified using "complete seqlinkage" command which works based on "Unweighted Pair Group Method with Arithmetic Mean" (UPGMA) (28). The analysis of homology between the miRNA sets of six lineages was also carried out by using InteractiVenn web tool (29).

4. Results

4.1. PPIs Network Analysis

After analyzing with DESeq, the DEGs of each cell lineage were chosen as genes that were significantly up-and down-regulated in differentiated cell samples compared with MSC samples. The non-functional genes (genes with no known function which are different from non-annotated genes) were removed and remained genes of each cell lineage were finally selected for constructing PPI networks. The number of nodes-edges in the PPI networks constructed from osteocyte, chondrocyte, adipocyte, myocyte, neurocyte and tenocyte gene expression profiles are 381-1724, 190-3026, 194-1855, 181-1397, 164-758 and 206-436 respectively. We analyzed the topological properties of interactions in the constructed PPI networks to determine key hub genes of MSCs differentiation. The results of nine performed methods were compared together and it turned out that Degree and Betweenness methods were highly reliable to identify the functional genes likely to be involved in the MSCs differentiation. However, based on the node degree distribution and neighborhood connectivity distribution charts, all constructed PPI networks showed a biological scale-free pattern. Node degree distribution chart indicated that many nodes had low numbers of interactions and a few nodes were highly connected. Neighborhood connectivity distribution showed an assortative pattern,

Table 1. Transcriptional regulatory hub genes resulted from functional annotation using DAVID tool.

Cell lineages					
Osteocyte	Chondrocyte	Adipocyte	Myocyte	Neurocyte	Tenocyte
KLF10	SMAD3	CEBPA	FOSL1	GLI2	GATA6
SMAD1	SOX9	DDIT3	SIX1	POU2F1	SMAD9
SMAD5	BMP2	E2F1	ACTN2	SIX1	BATF
SP1	EGR2	GATA2	BGLAP	SOX1	DLX2
SP7	FGF2	SOX11	DLX2	SOX2	EGR1
ATF4	FOXO1	BGLAP	DLX5	SOX5	ETV5
BMP2	FOXO3	BMP2	FOXC2	SOX6	LEF1
CTNNB1	IGF1	EBF1	FHL2	ASCL1	NFATC1
EGR2	IL6	FABP4	LBX1	BMP2	NR2F1
FOXM1	NFATC2	FOXM1	LEF1	CTNND1	PPARG
FOXO1	NFATC3	FOXO1	MSX1	CUX2	RUNX3
MSX1	PTH	LEF1	MEF2A	EGR2	BMP4
MSX2	TGFB1	NFYB	MEF2C	EYA2	FGF2
MEN1	GDF5	PITX1	MEF2D	FGF2	IGF1
NCOR2	NKX3-2	PPARG	MYOD1	GDNF	IL6
PPARG		PPARGC1A	MYF5	HES5	PAX1
PTTG1		PBX1	MYF6	HAND2	TGFB3
RUNX2		SIRT1	MYOG	HDAC9	TNMD
STAT3		SREBF1	PAX3	HOXA10	
TCF3		TCF7L2	PAX7	HOXA9	
TGFB1		MYBL2	RUNX2	HOXC6	
TWIST1			TGFB1	IGF1	
MYBL2			TWIST1	NEUROD1	
ZBTB16				NEUROG1	
				NEUROG2	
				NEUROG3	
				PAX6	
				PBX1	
				STAT3	
				STAT6	
				SPI1	
				TWIST1	

which means that nodes had a tendency to connect with nodes with similar connectivity (cytoscape exportable files are available in Supplementary PPI data).

After filtering and removing duplicate values between two applied measures, we obtained a large number of unique genes (143 from osteocyte, 138 from chondrocyte, 134 from adipocyte, 131 from myocyte, 128 from neurocyte and 142 from tenocyte) and defined these genes as the main candidate genes involved in MSCs differentiation (**supplementary Table S2**). Presence of several cell-specific biomarkers (i.e. RUNX2 and SP7 for osteocyte, SOX9 for chondrocyte, PPARG for adipocyte, MEF2A, 2C and 2D for myocyte, SOX2, NEUROG1, G2, G3 and D1 for neurocyte and ETV5 and FGF2 for tenocyte) indicate that the PPI networks are highly cell-specific. We performed functional annotation of obtained hub genes using DAVID tool and regarded transcriptional regulatory genes category consisting of TFs and non-TFs genes for further analysis **(Table 1)**. However, functional

Table 2. Overrepresented DAVID terms for the hub genes of the PPI network

Cell type	Terms	Count.	%	P-value*
Adipocyte	Cell cycle	15	11.5	2.7E-6
	Signal transduction	43	33.1	9.8E-6
	Transcription regulation	25	19.2	4.0E-3
	Phosphorylation	60	46.2	3.8E-2
	Differentiation	9	6.9	1.2E-2
Neurocyte	Differentiation	16	12.7	2.8E-7
	Transcription regulation	31	24.6	1.0E-5
	Signal transduction	38	30.2	2.5E-4
	Developmental proteins	21	16.7	1.3E-7
Osteocyte	Cell cycle	16	15.1	3.0E-8
	Phosphorylation	60	56.6	9.2E-5
	Signal transduction	34	32.1	1.8E-4
	Transcription regulation	25	23.6	1.9E-4
	Developmental proteins	11	10.4	1.0E-2
	Differentiation	8	7.5	1.3E-2
Myocyte	Developmental proteins	29	22.8	2.8E-12
	Differentiation	13	10.2	5.0E-5
	Transcription regulation	27	21.3	8.2E-4
Chondrocyte	Signal transduction	55	49.5	6.1E-15
	Phosphorylation	53	47.7	2.6E-2
	Developmental proteins	10	9.0	3.5E-2
	Differentiation	8	7.2	1.7E-2
Tenocyte	Signal transduction	53	40.8	1.6E-10
	Developmental proteins	15	11.5	1.9E-2
	Differentiation	11	8.5	1.0E-3
	Phosphorylation	62	47.7	1.4E-2

* This p-value is based on the EASE Score, a modified Fisher Exact P-Value, for gene-enrichment analysis. It ranges from 0 to 1. Fisher Exact P-Value = 0 represents perfect enrichment. Usually P-Value is equal or smaller than 0.05 to be considered strongly enriched in the annotation categories. We considered the maximum EASE score as 0.05.

annotation of obtained hub genes from PPI network revealed that these genes share some similar processes, such as cell cycle, differentiation, signal transduction, phosphorylation, transcription regulation, and so on **(Table 2).** Among DAVID terms, signal transduction and phosphorylation categories which consist of the highest proportion of DEGs seem to have key role in the differentiation of MSCs.

4.2. MiRNAs-PPI Network and Clustering Analyses

MiRNA-PPI regulatory networks were reconstructed by adding corresponding miRNAs to the PPI network of each cell lineage. The resulting networks of osteocyte, chondrocyte, adipocyte, myocyte, neurocyte and tenocyte consist of 637-1613, 632-1491, 654-1668, 627-1557, 602-1438 and 634-1588 nodes-edges respectively. Topological analysis of these regulatory networks identified a set of hub miRNAs for each cell type. The miRNAs with higher out-degree measure are tabulated in the **Table 3.** After removing duplicate miRNAs, we obtained 35 unique miRNAs identified as hub miRNAs in six networks and represented them along with their targets in the **Figure 3**. However, the expression levels of these hub miRNAs need to be experimentally evaluated to identify the up- and down-regulated hub miRNAs in each cell lineage.

Enrichment of all six lineage-related hub miRNAs indicated that some particular functional categories such as Cell cycle, Apoptosis, AKT signaling pathway, Angiogenesis, Hormones regulation, Human embryonic stem cell (hESC) regulation and Cell differentiation were significantly overrepresented **(Table 4)**. A comparison of predicted miRNA patterns of six cell lineages demonstrated that ten clusters of miRNAs including: has-let-7a, has-mir-15a, has-mir-181a, has-mir-182, has-mir-196a, has-mir-221, has-mir-29a, has-mir-29b, has-mir-30a and has-mir-374b are common in all six miRNA-PPI networks. The functional annotation of selected hub genes and miRNAs suggest that signal transduction and cell cycle related pathways play a pivotal role in the regulating MSCs differentiation. The enrichment of hub miRNAs revealed that a high number of miRNAs are participated in the modulation of AKT signaling pathway. The central role of AKT signaling

Table 3. The hub miRNAs obtained from topological analysis of miRNAs-gPPI network (Out-degree >10).

Cell lineages					
Osteocyte	Chondrocyte	Adipocyte	Myocyte	Neurocyte	Tenocyte
hsa-miR-335	hsa-miR-335	hsa-miR-335	hsa-miR-335	hsa-miR-335	hsa-miR-335
hsa-miR-26b	hsa-miR-34a	hsa-miR-124	hsa-miR-124	hsa-miR-124	hsa-miR-124
hsa-miR-34a	hsa-miR-124	hsa-miR-26b	hsa-miR-26b	hsa-miR-26b	hsa-miR-26b
hsa-miR-124	hsa-miR-26b	hsa-miR-34a	hsa-miR-1	hsa-miR-16	hsa-miR-98
hsa-miR-193b	hsa-miR-98	hsa-miR-193b	hsa-miR-16	hsa-miR-192	hsa-miR-1
hsa-let-7b	hsa-miR-1	hsa-miR-192	hsa-miR-155	hsa-miR-128	hsa-miR-92a
hsa-miR-192	hsa-let-7b	hsa-miR-215	hsa-miR-98	hsa-miR-1	hsa-miR-16
hsa-miR-16	hsa-miR-193b	hsa-let-7b	hsa-miR-193b	hsa-miR-155	hsa-let-7b
hsa-miR-215	hsa-miR-16	hsa-miR-98	hsa-let-7b	hsa-miR-98	hsa-miR-34a
hsa-miR-1	hsa-miR-92a	hsa-miR-484	hsa-miR-92a	hsa-miR-21	hsa-miR-193b
hsa-miR-484	hsa-miR-155	hsa-miR-615	hsa-miR-484	hsa-miR-7	hsa-miR-155
hsa-miR-92a	hsa-miR-615	hsa-miR-1	hsa-miR-34a	hsa-miR-215	hsa-miR-192
hsa-miR-155	hsa-miR-18a	hsa-miR-16	hsa-miR-125b	hsa-miR-125b	hsa-miR-21
hsa-miR-98	hsa-miR-21	hsa-miR-92a	hsa-miR-19	hsa-miR-615	hsa-miR-125b
hsa-miR-615	hsa-miR-484	hsa-miR-128	hsa-miR-7	hsa-miR-148b	hsa-miR-615
hsa-miR-320a	hsa-miR-320a	hsa-miR-148b	hsa-miR-615	hsa-miR-193b	hsa-miR-222
hsa-miR-186	hsa-miR-221	hsa-miR-155	hsa-miR-21	hsa-miR-93	hsa-miR-30a
hsa-miR-24	hsa-miR-192	hsa-miR-149	hsa-miR-30a	hsa-miR-92a	hsa-miR-146a
hsa-miR-21	hsa-let-7a	hsa-miR-196a			hsa-miR-148b
hsa-miR-125b		hsa-miR-24			hsa-miR-484
hsa-miR-30a		hsa-miR-21			hsa-let-7a
hsa-miR-149		hsa-miR-186			hsa-miR-24
hsa-miR-93		hsa-let-7a			hsa-miR-215
hsa-let-7a					hsa-miR-20a
hsa-miR-9					
hsa-miR-196a					
hsa-miR-17					

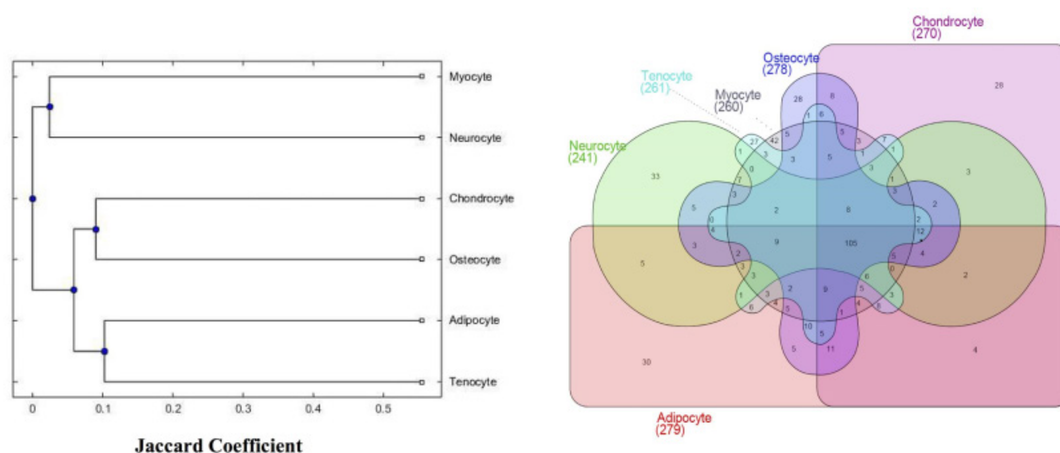

Figure 3. Dendrogram (left) and Venn diagram (right) summarizing the overlapping homology between six lineages miRNA pattern. Based on the possible miRNA expression patterns, the six lineages divided into two distinct groups.

Table 4. Overrepresented TAM functional categories in selected hub miRNAs

Functional categories	Osteocyte		Chondrocyte		Adipocyte		Myocyte		Neurocyte		Tenocyte	
	Count.	P-value	Count.	P-value	Count.	P-value	Count.	P-value	Count.	P-value	Count.	P-value
AKT pathway	10	2.34e-6	9	2.30e-6	8	3.92e-5	7	1.79e-4	5	6.83e-3	11	4.31e-8
Angiogenesis regulation	13	2.00e-7	9	7.64e-5	10	1.36e-5	10	4.81e-6	8	1.96e-4	15	1.71e-10
Apoptosis regulation	18	1.51e-7	13	2.83e-5	17	1.96e-8	14	1.90e-6	9	3.99e-3	18	1.78e-8
Cell cycle regulation	26	1.94e-10	22	6.76e-10	23	2.17e-10	20	1.03e-8	19	1.87e-8	26	6.43e-12
Cell differentiation	6	8.69e-3	6	2.08e-3	6	2.60e-3	5	9.25e-3	5	6.83e-3	7	7.54e-4
Hormones regulation	25	2.77e-10	17	3.37e-6	17	6.55e-6	18	1.74e-7	15	1.32e-5	22	6.46e-9
hESC regulation	27	2.50e-8	18	9.05e-5	21	2.18e-6	19	6.86e-6	19	1.87e-6	23	1.00e-6

pathway in the regulation of MSCs proliferation, survival, differentiation and migration has been reported more and more (30, 31). Lim and colleagues found that Akt-dependent up-regulation of Bcl2 mediates the differentiation and survival of MSCs stimulated by Brain-derived neurotrophic factor (BDNF) (32).

Owing to the fact that there are considerable differences between the miRNA expression signatures of MSC-derived lineages (10), miRNA-based classification may provide a deeper insight into the perception of common regulatory mechanisms underlying the self-renewal and differentiation of MSCs. In this step, cell lineages were classified only by considering the experimentally validated miRNAs corresponding to the DEGs. The six non-mesenchymal cell lineages were divided in two groups by the cluster analysis. The first group was composed of myocyte and neurocyte, while the second group contained chondrocyte, osteocyte, adipocyte and tenocyte. The results of the classification clearly indicated that the all six cell lineages share a large number of similar miRNAs. However, there was no previous study reporting the origin of studied cell lineages according to their miRNA expression profile, and therefore the details of this result must be validated experimentally.

5. Discussion

Previous studies have reported that canonical WNT/CTNNB1 signaling pathway plays a crucial role in the specific stages of osteogenesis by directly regulating RUNX2 gene expression (33). Here, we found CTNNB1 and TCF3 genes involved in canonical WNT pathway as hub genes of osteocyte PPI network. The KLF10, ATF4 and FOXO1 ,three hub genes of osteocyte, can also promote osteogenesis by stimulating RUNX2 expression (34, 35), whereas BMP2 and MSX2 can up-regulate SP7/OSX in the MSCs undergoing osteogenic differentiation (36).

LEF1 is a hub gene of adipocyte, myocyte and tenocyte PPI networks which participates in WNT signaling pathway. In has been revealed that WNT/LEF1 signaling pathway acts as positive regulator of myogenesis via up-regulation of PITX2 expression (37). The TGFβ signaling pathway is also necessary for differentiation of MSCs into particular lineages, especially osteocyte. Activation of TGFβ let to the up-regulation of RUNX2 and down-regulation of PPARG through interacting with PPARγ signaling pathway. This pathway provides a functional competence between osteogenesis and chondrogenesis during the early stages of their development and also inhibit differentiation of MSCs into adipocyte and myocyte (38). However, the detailed role of TGFβ signaling pathway in tenogenesis is not well described. We found some SMAD family members, involved in TGFβ signaling pathway, namely SMAD1, SMAD5, SMAD3 and SMAD9 as hub regulatory genes of osteo, chondro and tenocyte-derived PPI networks.

Recent studies have shown that two HOX and SOX families encode a highly conserve group of transcription factors which play a crucial role in regulating of lineage development. Our results indicated that several members of these two families (SOX1-6 and HOXC6, HOXA9, HOXA10) are obviously recognized as hub genes of neurocyte PPI network. The key role of SOX family in the regulation of neural development has been frequently reported (39). We found that SOX6 was significantly down-regulated in the neurocyte samples compared to MSC samples suggesting its negative effect on the neural development by maintaining the undifferentiated state of neural progenitors. In addition to neurocyte, we obtained SOX9 and SOX11 as hub genes of chondrocyte and adipocyte networks respectively. SOX9, a master regulator of chondrogenesis, is required for successive differentiation of MSCs into chondrogenic lineage (40). Overexpression of SOX11 has been observed in the MSCs undergoing osteogenesis, adipogenesis,

neurogenesis and chondrogenesis proposing that expression of this gene is critical for differentiation of MSCs into four mentioned lineages (41).
FGF2 is a member of growth factors that implicate in diverse biological processes, such as cell growth, cell differentiation and wound healing. This gene was found as a hub gene of tenocyte, neurocyte and chondrocyte PPI networks. FGF2 enhances the chondrogenic potential of MSCs through strong induction of SOX9 expression (42). This gene sufficiently increases tenogenesis by activating ETV4 and ETV5 transcription factors that result in the induction of SCX, a tendon-specific marker (43). FGF2 can also indirectly inhibit differentiation of MSCs into neural lineage via interaction with NOTCH signaling pathway (44). TWIST1, a hub gene of osteocyte, myocyte and neurocyte PPI networks, plays a crucial role in the regulation of osteogenesis (45) and muscle development (46). However, its function in neurogenesis has not yet been established. EGR1 and EGR2 are transcriptional regulators that activate the expression of several genes whose products are required for adipogenic and tenogenic differentiation of stem cells (47, 48). The EGFR-induced expression of EGR2 can also preserve the capacity of osteoprogenitor proliferation (49). SIRT1 was distinguished as the most important hub gene of adipocyte PPI network suggesting its key role in promoting adipogenesis and adipose tissue development. Recent studies have demonstrated that SIRT1 can induce the expression of ADIPOQ, an adipose-derived hormone expressed during the early phase of adipogenesis, by enhancing the FOXO1 and CEBPA interaction (50).
It has been observed that miRNA expression profile of MSC is changed considerably during differentiation into cell lineages. The corresponding functionally active miRNAs are involved in the regulation of specific stages of MSCs differentiation. Among the all six lineages miRNAs-PPI networks, hsa-miR-335 was found as a hub miRNA with the highest out-degree. Then, this miRNA considered as the most important hub miRNA in the analyzed miRNAs-PPI networks. Down-regulation of hsa-miR-335 has been experimentally observed upon MSCs differentiation. Recent reports have indicated that overexpression of hsa-miR-335 can inhibit the differentiation and migration of human MSCs (51). The expression of has-miR-335 in MSCs activates WNT signaling pathway that results in the increased stemness, and the acquisition of migration capability (52). This miRNA regulate proliferation and differentiation of MSCs through targeting high number of genes, almost 60 genes, such as RB1 and RUNX2 (51, 53). The expression of two identified hub miRNAs including has-miR-124 and has-miR-34a is also associated with differentiation of MSCs. Ectopic expression of has-miR-124 resulted in the substantially down-regulation of ACAN, a chondrocyte marker, as well as the up-regulation of an adipocyte marker, FABP4 (54). In the neural precursor cells, has-miR-34a is able to control MSCs neurogenesis through modulating the genes involved in the regulation of cell motility (55). This miRNA also acts as negative regulator of osteogenesis by inhibiting translation of JAG1 (56). JAG1 is a ligand for NOTCH receptors mediating NOTCH signaling pathway and is able to increase osteogenic potential of MSCs (57). Hsa-miR-26 family consisting of hsa-miR-26a and hsa-miR-26b are actively participating in the regulation of MSCs differentiation. This family can facilitate the MSCs osteogenesis possibly by targeting some osteogenesis inhibitory genes such as HDAC4 and CDK6 (58). Furthermore, hsa-miR-26b is also known to act as a negative regulator of adipocytes differentiation, likely by regulating PTEN expression (59). The hsa-let-7 family is another important family of miRNAs which is well known because of its tumor suppressor activity. Moreover, the members of this family led to a temporal switch of MSCs from undifferentiated state to various differentiated cell lineages (60). Except for neurocyte network, we obtained hsa-let-7a and hsa-let-7b as hub miRNA of all analyzed networks. The specific role of hsa-let-7 family in the promotion of osteogenesis is mediated through regulation of HMGA2 (61).
Our results indicated that hsa-miR-148b is a hub miRNA of neurocyte, tenocyte and adipocyte miRNAs-PPI networks. Members of hsa-mir-148 family, which include hsa-miR148a, hsa-miR-148b and hsa-miR-152, have an aberrant expression in different stages of MSC differentiation. The experimental studies, have also reported that this family is involved in the regulation of osteogenesis, myogenesis and adipogenesis (62). Schoolmeesters et al. found that the expression of hsa-miR-148b significantly increased during early steps of osteogenesis (63). Overexpression of hsa-miR-148b has also reported in the mouse adipocytes and referred as a high confidence PPARG target (64). PPARG is a negative regulator of osteogenesis and inhibition of this gene can efficiently promote the osteogenesis of MSCs. The hsa-miR-20, as one of the identified adipocyte hub miRNA, can accelerate the differentiation of MSCs into osteocytes through targeting PPARG (65). In all six miRNA-PPI networks, hsa-miR-21 was recognized as an important hub miRNA (Out-degree>10). Hsa-miR-21 is able to inhibit osteogenesis and promote adipogenesis by modulating TGFβ signaling pathway (66). However,

Yang Mei *et al*. reported that overexpression of hsa-miR-21 can enhance the expression of PPARG and RUNX2 resulting in an increased in adipogenesis and osteogenesis respectively (67).

6. Conclusion

Our results also introduced FOXO1 as a down-regulated hub gene of chondrocyte and adipocyte PPI networks. FOXO1 suppresses adipocyte differentiation by repressing the expression of PPARG gene (68). In this study, some other FOX family members including FOXM1, FOXC2 and FOXO3 were also determined as hub regulatory genes of osteocyte, chondrocyte, adipocyte and myocyte networks. Recently, a considerable attention has been focused on studying the roles of FOX family in cancer progression and invasion. More details are given in Ref (69). According to our results, this family of transcription factors can be considered as new potential candidates for *in vitro* manipulation of MSCs.

The HOX family, either transcriptionally or epigenetically, regulates neurogenesis in the vertebrates. However, the possible mechanism underlying transcriptional regulation of HOX genes during neural development is barely understood. There were two other homeobox-containing genes including DLX2 and DLX5 in the final lists of tenocyte and myocyte hub genes. It has been shown that DLX2 negatively regulates NOTCH signaling pathway and DLX5 inhibits neural differentiation of MSCs (70). The NOTCH signaling pathway functions in the sustaining stemness of MSCs as well as neural lineage development. Based upon these findings, we suggest that DLX2/5 can promote myogenesis by inhibiting NOTCH signaling pathway and consequently suppressing neurogenesis. However, we couldn't find any published data explaining the specific role of DLX proteins in the regulation of tenogenesis.

The role of hsa-miR-192 in the regulation of MSCs differentiation is not well established and researchers have mainly focused on its function in the cancer progression (71). However, it has been reported that PPARG gene expression, a marker of adipocyte, is regulated by hsa-miR-192 induction (72). The inhibition or induction of hsa-miR-9 and hsa-miR-98 can regulate IL1B, TNFA, MMP13 and COL2A1 genes expression indicating their important roles in the development of chondrocytes and osteocytes (73, 74).

In conclusion, we aimed to identify key regulatory genes and miRNAs likely to be involved in the regulation of MSCs differentiation. Consistent with recent publications, we found a set of hub genes and miRNAs seemed to be key regulators of MSCs differentiation. Our results also introduced a number of new regulatory genes and miRNAs which can be considered as the new candidates for genetic *manipulation of MSCs.*

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This research is supported by Research Core: "Bio-Mathematics with computational approach" of Tarbiat Modares University, with grant number "IG-39706" and the Russian Government Program of Competitive Growth of Kazan Federal University. Albert A. Rizvanov was supported by state assignments 20.5175.2017/6.7 and 17.9783.2017/8.9 of the Ministry of Science and Higher Education of Russian Federation.

References

1. Bernardo ME, Fibbe WE. Safety and efficacy of mesenchymal stromal cell therapy in autoimmune disorders. *Ann N Y Acad Sci.* 2012;**1266**(1):107-117. doi:10.1111/j.1749-6632.2012.06667.x
2. Huang Y, Enzmann V, Ildstad ST. Stem cell-based therapeutic applications in retinal degenerative diseases. *Stem Cell Rev Rep*. 2011;**7**(2):434-445. doi:10.1007/s12015-010-9192-8
3. Shah K. Mesenchymal stem cells engineered for cancer therapy. *Adv Drug Deliv Rev*. 2012;**64**(8):739-748. doi:10.1016/j.addr.2011.06.010
4. Zhu L, Del Vecchio G, De Micheli G, Liu Y, Carrara S, Calzà L, *et al.* Biochips for regenerative medicine: Real-time stem cell continuous monitoring as inferred by high-throughput gene analysis. *BioNanoScience*. 2011;**1**(4):183-191. doi:10.1007/s12668-011-0028-z
5. Yang L, Wang J, Wang H, Lv Y, Zuo Y, Jiang W. Characterization of essential genes by topological properties in the perturbation sensitivity network. *Biochem Biophys Res Commun*. 2014;**448**(4):473-479.
6. Maryam I, Seyede SM, Seyed SA, Sadegh Azimzadeh SA, Sadegh A, Mansour P. HOXB7 and Hsa-miR-222 as the Potential Therapeutic Candidates for Metastatic Colorectal Cancer. *Recent Pat Anticancer Drug Discov.* 2016;**11**:1-10. doi: 10.2174/1574892811999160628114857
7. Ataei A, Solovyeva VV, Poorebrahim M, Blatt NL, Salafutdinov II, Şahin F, *et al*. A Genome-Wide Analysis of mRNA Expression in Human Tooth Germ Stem Cells Treated with Pluronic P85. *BioNanoScience*. 2016:1-11. doi:10.1007/s12668-016-0254-5
8. Sengupta D, Bandyopadhyay S. Topological patterns in microRNA–gene regulatory network: studies in colorectal and breast cancer. *Mol Biosyst*. 2013;**9**(6):1360-1371. doi:10.1039/c3mb25518b
9. Guo L, Zhao RC, Wu Y. The role of microRNAs in self-renewal and differentiation of mesenchymal stem cells. *Exp Hematol.* 2011;**39**(6):608-616. doi:10.1016/j.exphem.2011.01.011
10. Gao J, Yang T, Han J, Yan K, Qiu X, Zhou Y, *et al*. MicroRNA expression during osteogenic differentiation of human multipotent mesenchymal stromal cells from bone marrow. *J Cell Biochem*. 2011;**112**(7):1844-1856. doi:10.1002/jcb.23106
11. Crobu F, Latini V, Marongiu MF, Sogos V, Scintu F, Porcu S, *et al*.

Differentiation of single cell derived human mesenchymal stem cells into cells with a neuronal phenotype: RNA and microRNA expression profile. *Mol Biol Reports*. 2012;**39**(4):3995-4007. doi:10.1007/s11033-011-1180-9 12.
12. Huang J, Zhao L, Xing L, Chen D. MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. *Stem Cells*. 2010;**28**(2):357-364. doi: 10.1002/stem.288
13. Yang B, Guo H, Zhang Y, Chen L, Ying D, Dong S. MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. *PLoS One*. 2011;**6**(7):e21679. doi:10.1371/journal.pone.0021679
14. Esau C, Kang X, Peralta E, Hanson E, Marcusson EG, Ravichandran LV, *et al*. MicroRNA-143 regulates adipocyte differentiation. *J Biol Chem*. 2004;**279**(50):52361-52365. doi:10.1074/jbc.c400438200
15. Cheng L-C, Pastrana E, Tavazoie M, Doetsch F. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat Neurosci*. 2009;**12**(4):399-408. doi:10.1038/nn.2294
16. Ivey KN, Srivastava D. MicroRNAs as regulators of differentiation and cell fate decisions. *Cell Stem Cell.* 2010;**7**(1):36-41. doi: 10.1016/j.stem.2010.06.012
17. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, *et al*. MicroRNA expression profiles classify human cancers. *Nature*. 2005;**435**(7043):834-838. doi: 10.1038/nature03702
18. Lizio M, Harshbarger J, Shimoji H, Severin J, Kasukawa T, Sahin S, *et al*. Gateways to the FANTOM5 promoter level mammalian expression atlas. *Genome biolog*. 2015;**16**(1):22.
19. Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biol*. 2010;**11**(10):R106. doi: 10.1186/gb-2010-11-10-r106
20. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, *et al*. STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res*. 2013;**41**(D1):D808-D815. doi: 10.1093/nar/gks1094
21. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, *et al*. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res*. 2010;**38**(suppl 2):W214-W220. doi:10.1093/nar/gkq537
22. Zhao F, Xuan Z, Liu L, Zhang MQ. TRED: a Transcriptional Regulatory Element Database and a platform for in silico gene regulation studies. *Nucleic Acids Res*. 2005;**33**(suppl 1):D103-D117. doi:10.1093/nar/gki004
23. Hsu S-D, Lin F-M, Wu W-Y, Liang C, Huang W-C, Chan W-L, *et al*. miRTarBase: a database curates experimentally validated microRNA–target interactions. *Nucleic Acids Res*. 2010:gkq1107. doi: 10.1093/nar/gkq1107
24. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;**13**(11):2498-2504. doi:10.1101/gr.1239303
25. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2008;**4**(1):44-57. doi:10.1038/nprot.2008.211
26. Giovannetti E, Funel N, Peters GJ, Del Chiaro M, Erozenci LA, Vasile E, *et al*. MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity. *Cancer Res*. 2010;**70**(11):4528-4538. doi:10.1158/0008-5472.can-09-4467
27. Finch H. Comparison of distance measures in cluster analysis with dichotomous data. *J Data Sci*. 2005;**3**(1):85-100.
28. Henson R, Cetto L. The MATLAB bioinformatics toolbox. Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics. 2004. doi:10.1002/047001153x.g409308
29. Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*. 2015;**16**(1):169. doi:10.1186/s12859-015-0611-3
30. Chen J, Crawford R, Chen C, Xiao Y. The key regulatory roles of the PI3K/Akt signaling pathway in the functionalities of mesenchymal stem cells and applications in tissue regeneration. *Tissue Eng Part B Rev*. 2013;**19**(6):516-528. doi:10.1089/ten.teb.2012.0672
31. Ryu CH, Park SA, Kim SM, Lim JY, Jeong CH, Jun JA, *et al*. Migration of human umbilical cord blood mesenchymal stem cells mediated by stromal cell-derived factor-1/CXCR4 axis via Akt, ERK, and p38 signal transduction pathways. *Biochem Biophys Res Commun*. 2010;**398**(1):105-110. doi:10.1016/j.bbrc.2010.06.043
32. Lim JY, Park SI, Oh JH, Kim SM, Jeong CH, Jun J, *et al*. Brain-derived neurotrophic factor stimulates the neural differentiation of human umbilical cord blood-derived mesenchymal stem cells and survival of differentiated cells through MAPK/ERK and PI3K/Akt-dependent signaling pathways. *J Neurosci Res*. 2008;**86**(10):2168-2178. doi:10.1002/jnr.21669
33. Gaur T, Lengner CJ, Hovhannisyan H, Bhat RA, Bodine PV, Komm BS, *et al*. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. *J Biol Chem*. 2005;**280**(39):33132-33140. doi:10.1074/jbc.m500608200
34. Hawse JR, Cicek M, Grygo SB, Bruinsma ES, Rajamannan NM, Van Wijnen AJ, *et al*. TIEG1/KLF10 modulates Runx2 expression and activity in osteoblasts. *PloS one*. 2011;**6**(4):e19429. doi:10.1371/journal.pone.0019429
35. Lin KL, Chou CH, Hsieh SC, Hwa SY, Lee MT, Wang FF. Transcriptional upregulation of DDR2 by ATF4 facilitates osteoblastic differentiation through p38 MAPK-mediated Runx2 activation. *J Bone Miner Res*. 2010;**25**(11):2489-2503. doi: 10.1002/jbmr.159
36. Cheng S-L, Shao J-S, Charlton-Kachigian N, Loewy AP, Towler DA. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. *J Biol Chem*. 2003;**278**(46):45969-45977. doi:10.1074/jbc.m306972200
37. Abu-Elmagd M, Robson L, Sweetman D, Hadley J, Francis-West P, Münsterberg A. Wnt/Lef1 signaling acts via Pitx2 to regulate somite myogenesis. *Dev Bio*. 2010;**337**(2):211-219. doi:10.1016/j.ydbio.2009.10.023
38. Roelen BA, Ten Dijke P. Controlling mesenchymal stem cell differentiation by TGFβ family members. *J Orthopaedic Sci*. 2003;**8**(5):740-748. doi:10.1007/s00776-003-0702-2
39. Pevny L, Placzek M. SOX genes and neural progenitor identity. *Curr Opin Neurobiol*. 2005;**15**(1):7-13. doi:10.1016/j.conb.2005.01.016
40. Akiyama H, Chaboissier M-C, Martin JF, Schedl A, de Crombrugghe B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6.

Genes Dev. 2002;**16**(21):2813-2828. doi:10.1101/gad.1017802

41. Xu L, Huang S, Hou Y, Liu Y, Ni M, Meng F, *et al*. Sox11-modified mesenchymal stem cells (MSCs) accelerate bone fracture healing: Sox11 regulates differentiation and migration of MSCs. *FASEB J*. 2015;**29**(4):1143-1152. doi:10.1096/fj.14-254169
42. Murakami S, Kan M, McKeehan WL, De Crombrugghe B. Up-regulation of the chondrogenic Sox9 gene by fibroblast growth factors is mediated by the mitogen-activated protein kinase pathway. *Proc Natel Acad Sci U S A*. 2000;**97**(3):1113-1118. doi:10.1073/pnas.97.3.1113
43. Brent AE, Tabin CJ. FGF acts directly on the somitic tendon progenitors through the Ets transcription factors Pea3 and Erm to regulate scleraxis expression. *Development*. 2004;**131**(16):3885-3896. doi:10.1242/dev.01275
44. Faux CH, Turnley AM, Epa R, Cappai R, Bartlett PF. Interactions between fibroblast growth factors and Notch regulate neuronal differentiation. *J Neuroscience*. 2001;**21**(15):5587-5596. doi:10.1523/jneurosci.21-15-05587.2001
45. Yousfi M, Lasmoles F, Kern B, Marie PJ. TWIST inactivation reduces CBFA1/RUNX2 expression and DNA binding to the osteocalcin promoter in osteoblasts. *Biochem Biophys Res Commun*. 2002;**297**(3):641-644. doi:10.1016/s0006-291x(02)02260-x
46. Koutsoulidou A, Mastroyiannopoulos NP, Furling D, Uney JB, Phylactou LA. Endogenous TWIST expression and differentiation are opposite during human muscle development. *Muscle Nerve*. 2011;**44**(6):984-986. doi:10.1002/mus.22241
47. Ahmed M, Gaffen SL. IL-17 inhibits adipogenesis in part via C/EBPα, PPARγ and Krüppel-like factors. *Cytokine*. 2013;**61**(3):898-905. doi:10.1016/j.cyto.2012.12.007
48. Tao X, Liu J, Chen L, Zhou Y, Tang K. EGR1 Induces Tenogenic Differentiation of Tendon Stem Cells and Promotes Rabbit Rotator Cuff Repair. *Cell Physiol Biochem*. 2015;**35**(2):699-709. doi:10.1159/000369730
49. Chandra A, Lan S, Zhu J, Siclari VA, Qin L. Epidermal growth factor receptor (EGFR) signaling promotes proliferation and survival in osteoprogenitors by increasing early growth response 2 (EGR2) expression. *J Biol Chem*. 2013;**288**(28):20488-20498. doi:10.1074/jbc.m112.447250
50. Qiao L, Shao J. SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein α transcriptional complex. *J Biol Chem*. 2006;**281**(52):39915-39924. doi:10.1074/jbc.m607215200
51. Tomé M, López-Romero P, Albo C, Sepúlveda JC, Fernández-Gutiérrez B, Dopazo A, *et al*. miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells. *Cell Death Differ*. 2011;**18**(6):985-995. doi:10.1038/cdd.2010.167
52. Zhang J, Tu Q, Bonewald LF, He X, Stein G, Lian J, *et al*. Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. *J Bone Mineral Res*. 2011;**26**(8):1953-1963. doi:10.1002/jbmr.377
53. Scarola M, Schoeftner S, Schneider C, Benetti R. miR-335 directly targets Rb1 (pRb/p105) in a proximal connection to p53-dependent stress response. *Cancer Res*. 2010;**70**(17):6925-6933. doi:10.1158/0008-5472.can-10-0141
54. Laine SK, Alm JJ, Virtanen SP, Aro HT, Laitala-Leinonen TK. MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. *J cell biochem*. 2012;**113**(8):2687-2695. doi:10.1002/jcb.24144
55. Chang S-J, Weng S-L, Hsieh J-Y, Wang T-Y, Chang MD, Wang H-W. MicroRNA-34a modulates genes involved in cellular motility and oxidative phosphorylation in neural precursors derived from human umbilical cord mesenchymal stem cells. *BMC Med Genomics*. 2011;**4**(1):65. doi:10.1186/1755-8794-4-65
56. Chen L, Holmstrøm K, Qiu W, Ditzel N, Shi K, Hokland L, *et al*. MicroRNA-34a Inhibits Osteoblast Differentiation and In Vivo Bone Formation of Human Stromal Stem Cells. *Stem cells*. 2014;**32**(4):902-912. doi:10.1002/stem.1615
57. Dishowitz MI, Zhu F, Sundararaghavan HG, Ifkovits JL, Burdick JA, Hankenson KD. Jagged1 immobilization to an osteoconductive polymer activates the Notch signaling pathway and induces osteogenesis. *J Biomed Mater Res A*. 2014;**102**(5):1558-1567. doi:10.1002/jbm.a.34825
58. Trompeter H-I, Dreesen J, Hermann E, Iwaniuk KM, Hafner M, Renwick N, *et al*. MicroRNAs miR-26a, miR-26b, and miR-29b accelerate osteogenic differentiation of unrestricted somatic stem cells from human cord blood. *BMC genomics*. 2013;**14**(1):111. doi:10.1186/1471-2164-14-111
59. Song G, Xu G, Ji C, Shi C, Shen Y, Chen L, *et al*. The role of microRNA-26b in human adipocyte differentiation and proliferation. *Gene*. 2014;**533**(2):481-487. doi:10.1016/j.gene.2013.10.011
60. Koh W, Sheng CT, Tan B, Lee QY, Kuznetsov V, Kiang LS, *et al*. Analysis of deep sequencing microRNA expression profile from human embryonic stem cells derived mesenchymal stem cells reveals possible role of let-7 microRNA family in downstream targeting of hepatic nuclear factor 4 alpha. *BMC genomics*. 2010;**11**(Suppl 1):S6. doi:10.1186/1471-2164-11-s1-s6
61. Wei J, Li H, Wang S, Li T, Fan J, Liang X, *et al*. let-7 enhances osteogenesis and bone formation while repressing adipogenesis of human stromal/mesenchymal stem cells by regulating HMGA2. *Stem Cells Dev*. 2014;*23*(13):1452-1463. doi:10.1089/scd.2013.0600
62. Chen Y, Song Y-X, Wang Z-N. The microRNA-148/152 family: multi-faceted players. *Mol Cancer*. 2013;**12**(1):43. doi:10.1186/1476-4598-12-43
63. Schoolmeesters A, Eklund T, Leake D, Vermeulen A, Smith Q, Aldred SF, *et al*. Functional profiling reveals critical role for miRNA in differentiation of human mesenchymal stem cells. *PLoS One*. 2009. doi: 10.1371/journal.pone.0005605
64. John E, Wienecke-Baldacchino A, Liivrand M, Heinäniemi M, Carlberg C, Sinkkonen L. Dataset integration identifies transcriptional regulation of microRNA genes by PPARγ in differentiating mouse 3T3-L1 adipocytes. *Nucleic Acids Res*. 2012;**40**(10):4446-4460. doi:10.1093/nar/gks025
65. Zhang J-f, Fu W-m, He M-l, Xie W-d, Lv Q, Wan G, *et al*. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. *RNA Biol*. 2011;**8**(5):829-838. doi: doi.org/10.1002/stem.23510.4161/rna.8.5.16043
66. Kim YJ, Hwang SJ, Bae YC, Jung JS. MiR-21 regulates adipogenic differentiation through the modulation of TGF-β signaling in mesenchymal stem cells derived from human adipose tissue. *Stem Cells*. 2009;**27**(12):3093-3102. doi:10.1002/stem.235
67. Mei Y, Bian C, Li J, Du Z, Zhou H, Yang Z, *et al*. miR-21 modulates the ERK–MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell

differentiation. *J Cell Biochem*. 2013;**114**(6):1374-84. doi:10.1002/jcb.24479
68. Gross D, van den Heuvel A, Birnbaum M. The role of FoxO in the regulation of metabolism. *Oncogene*. 2008;**27**(16):2320-2336. doi:10.1038/onc.2008.25
69. Katoh M, Katoh M. Human FOX gene family (Review). *Int J Oncolog*. 2004;**25**(5):1495-1500.
70. Yun K, Fischman S, Johnson J, de Angelis MH, Weinmaster G, Rubenstein JL. Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon. *Development*. 2002;**129**(21):5029-5040.
71. Sun Y, Pan J, Mao S, Jin J. IL-17/miR-192/IL-17Rs regulatory feedback loop facilitates multiple myeloma progression. *PLoS One*. 2014;**9**(12). doi:10.1371/journal.pone.0114647
72. Georges SA, Biery MC, Kim SY, Schelter JM, Guo J, Chang AN, *et al*. Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. *Cancer Res*. 2008;**68**(24):10105-10112. doi:10.1158/0008-5472.can-08-1846
73. Jones S, Watkins G, Le Good N, Roberts S, Murphy C, Brockbank S, *et al*. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-α and MMP13. *Osteoarthr. Cartil.* 2009;**17**(4):464-472. doi:10.1016/j.joca.2008.09.012
74. Dong S, Yang B, Guo H, Kang F. MicroRNAs regulate osteogenesis and chondrogenesis. *Biochem Biophys Res Commun*. 2012;**418**(4):587-591. doi:10.1016/j.bbrc. 2012. 01.075