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Identification of key pathways and genes in miR-30e regulating osteogenesis in aortic smooth muscle cells using bioinformatics analysis

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Abstract

Background: Cardiovascular disease (CVD) is an important problem that threatens the health of all mankind and remains the world's first cause of morbidity and mortality. In this study, we identified the gene characteristic during vascular calcification and explored their potential mechanisms.

Results: Gene expression profiles of GSE65435 were downloaded from GEO database. The GSE65435 dataset contained 18 samples, the 3 SMCs + miR-30e samples and 3 SMCs + ct-miR samples were selected. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analyses were implemented, and gene signal network and pathway relation network of the differentially expressed genes (DEGs) were analyzed by GCBI. In total, 620 differentially expressed genes were identified in SMCs + miR-30e samples, including 249 up-regulated genes and 371 down-regulated genes. GO analysis summed up that DEGs were significantly concentrated in negative regulation of apoptotic process. KEGG pathway analysis showed that DEGs were enriched in PI3K-Akt signaling pathway.

Conclusions: This study showed that the identified DEGs increased the molecular mechanisms underlying the development of vascular calcification and might be used as new diagnostic and therapeutic strategies for the treatment of vascular calcification.

Introduction

According to the World Health Organization (WHO) 2011 report, cardiovascular disease (CVD) is the primary problem that threatens the health of all mankind and remains the world's first cause of morbidity and mortality [1]. The number of cardiovascular diseases exceeds cancer, diabetes, chronic respiratory disease, etc. [2]. The data for 2008 showed that 30% of the total number of deaths worldwide are due to cardiovascular diseases, the vast majority of which were coronary heart diseases and stroke, and this trend is expected to continue [3]. In 2030, the number of death due to heart disease and stroke may increase from 18 million to 23.3 million and cardiovascular disease will continue to be the major cause of death [4]. In China, according to the China's health statistics released by the ministry of health, a total of 27,160,00 people died of cardiovascular diseases in 2007, becoming the first cause of death [5]. CVD pathogenesis has been a hot topic in the field of cardiovascular research, it has been commonly recognized that smooth muscle cell proliferation, inflammatory mediators, vascular endothelial dysfunction and lipid deposition are involved, among which vascular calcification plays an important role in the occurrence and development of a variety of cardiovascular diseases such as coronary heart disease, hypertension, degenerative heart valvular disease and cardiomyopathy [6]. Cardiovascular calcification is similar to the bone formation during the embryonic period, a variety of genes and proteins in minerals and bone metabolism have a certain regulatory role in vascular calcification [7].

MicroRNA (miRNA) is very important for gene regulation. Recent studies have shown that some miRNA can affect the process of bone formation by regulating the target genes, and could also have a regulatory role in the process of vascular calcification [8]. MiR-30e was reported to have the ability to induce adipogenic differentiation and reduce bone formation differentiation in stromal cells by targeting Lrp6 [9]. The article "miR-30e targets IGF2-regulated osteogenesis in bone marrow-derived mesenchymal stem cells, aortic smooth muscle cells, and ApoE2/2 mice" aimed to examine the role of miR-30e in vascular calcification. This article concluded that miR-30e inhibited the osteogenesis in MSCs and SMCs by targeting IGF2 and suppressed their differentiation into adipogenic or smooth muscle lineage [10]. In this study, there is an experiment that SMCs were treated with ct-miR or miR-30e and draw a conclusion that miR-30e inhibited the osteogenesis in MSCs and SMCs and suppressed their differentiation into adipogenic or smooth muscle lineage. The results of gene sequencing of two groups mice were upload to GEO DataSets (GSE65435) [11]. To further show the function of miR-30e of SMCs at the molecular level and explore the possible candidate biomarkers for diagnosis, prognosis, and drug targets, we are analyzing their biological functions and pathways.

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Key words: *Vascular calcification; miR-30e; differentially expressed genes (DEGs); gene ontology (GO); Kyoto Encyclopedia of Genes and Genomes pathway (KEGG)*

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Materials and methods

Microarray data

Gene expression profiles of GSE65435 were downloaded from the GEO database. GSE65435, which was based on Affymetrix GPL6246 platform (Affymetrix Mouse Gene 1.0 ST Array), was submitted by Wen, *et al*. The GSE65435 dataset comprised 18 samples. In this study, we selected the 6 samples, describing the effect of miR-30e in aortic smooth muscle cells differentiation.

Identification of DEGs

The raw data files were uploaded to the website of Gene-Cloud of Biotechnology Information (GCBI) [12]. SMCs + miR-30e samples were used as the test group and the SMCs + ct-miR used as the control group in this data. In the analysis process, we defined P = 0.05, Q = 0.05 and fold change = 2.0.

Gene ontology and pathway enrichment analysis of DEGs and network analysis

All the analysis was completed by the GCBI. In the gene ontology analysis, FDR = 0.05 and P = 0.05. In the pathway enrichment analysis, P = 0.05. Then we analyzed gene signal network to gene ontology analysis and analyzed pathway relation network to pathway enrichment. Then, networks were built by the cytoscape (3.4.0) software.

Results

Identification of DEGs

The samples were composed by 3 test samples and 3 control samples. Based on the $P < 0.05$ and fold control (FC) > 2.0 standards, a total of 620 genes were identified after the analyses of GSE65435, of which 249 were up-regulated and 371 were down-regulated (Figure 1). The gene Saa3 had a largest difference between SMCs + miR-30e group and SMCs + ct-miR group. The expression of gene Saa3 in SMCs + miR-30e group is 199.97 times in SMCs + ct-miR group. As showed in Table 1, the top ten DEGs were Saa3, Serpine2, Cd34, Grem1, Chi3l1, Hp, Selp, Ch25h, Clmp and Sfrp1.

GO term enrichment and KEGG pathway analysis

We analyzed DEGs by GCBI online tools which are mainly based on the algorithms of miR and target scan, to identify over represented GO categories and KEGG pathways. GO analysis indicated that up-regulated DEGs were significantly concentrated in biological processes (BP), including the cell cycle, cell division, and cell proliferation.

To identify the biological functions of these genes, GO and pathway enrichment analysis were implemented, respectively. As illustrated in Figure 2, the top ten regulated GOs sensitive to high concentration of miR-30e were positive regulation of angiogenesis, inflammatory response, positive regulation of apoptotic process, negative regulation

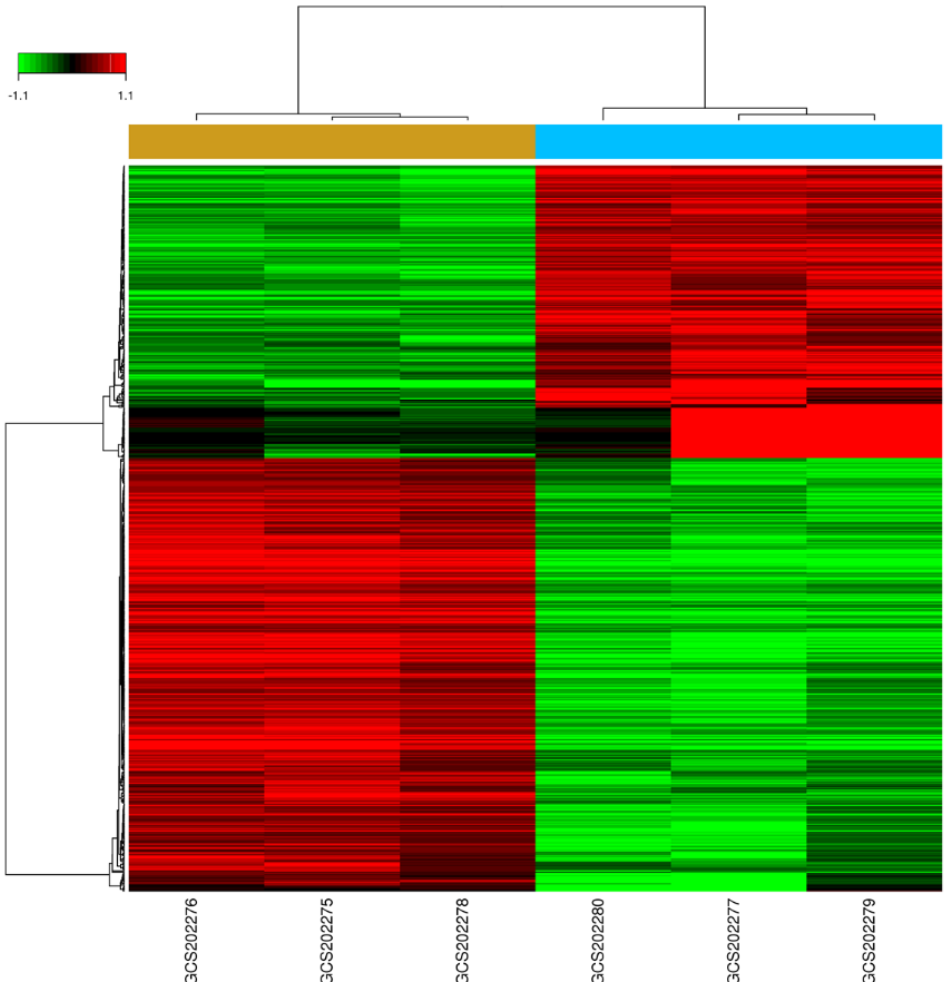

Figure 1. Changes in mRNAs expression profiles in miR-30e treated mice.

Table 1. The top 10 mRNAs with high degrees of DEGs.

Gene Symbol	Accession Number	Gene Description	d Score	Fold Change	Gene Feature
Saa3	NM_011315	Mus musculus serum amyloid A 3 (Saa3), mRNA	-31.68102	-199.976269	down
Serpine2	NM_009255	Mus musculus serine (or cysteine) peptidase inhibitor, clade E, member 2 (Serpine2), mRNA	-28.431854	-55.253872	down
Cd34	NM_001111059	Mus musculus CD34 antigen (Cd34), transcript variant 1, mRNA	-19.894471	-12.007931	down
Grem1	NM_011824	Mus musculus gremlin 1 (Grem1), mRNA	-19.045846	-20.450459	down
Chi3l1	NM_007695	Mus musculus chitinase 3-like 1 (Chi3l1), mRNA	-18.246297	-13.716394	down
Hp	NM_017370	Mus musculus haptoglobin (Hp), mRNA	-17.482949	-30.85244	down
Selp	NM_011347	Mus musculus selectin, platelet (Selp), mRNA	-15.500173	-8.72038	down
Ch25h	NM_009890	Mus musculus cholesterol 25-hydroxylase (Ch25h), mRNA	-15.265039	-22.893805	down
Clmp	NM_133733	Mus musculus CXADR-like membrane protein (Clmp), mRNA	-15.021017	-11.558135	down
Sfrp1	NM_013834	Mus musculus secreted frizzled-related protein 1 (Sfrp1), mRNA	-14.351045	-46.482443	down

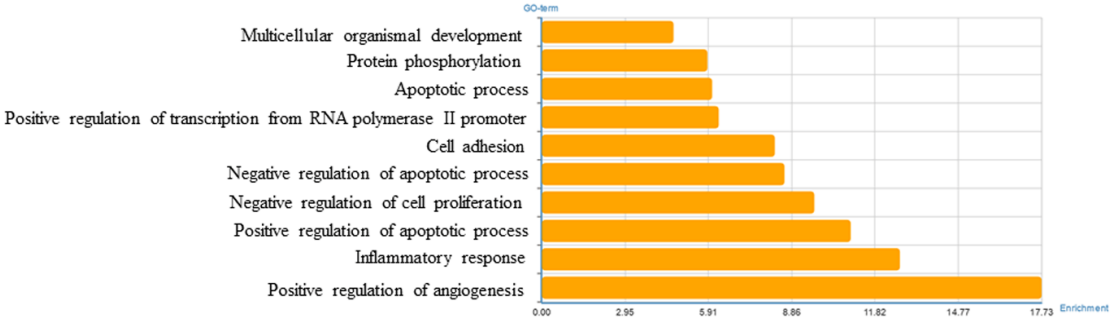

Figure 2. Significantly changed GOs of predicted target genes.

of cell proliferation, negative regulation of apoptotic process, cell adhesion, positive regulation of transcription from RNA polymerase II promoter, apoptotic process, protein phosphorylation and multicellular organismal development. And the genes of each function were listed in Table 2. GO analysis obviously suggested that high concentration of miR-30e could affect expression of many miRNAs, through many crucial functions such as regulation of angiogenesis, inflammatory response and positive regulation of apoptotic process of the mice with high expression of miR-30e. Combining with the KEGG database, we analyzed the pathways in which the putative target genes were involved. As illustrated in Figure 3, the top ten deregulated pathways sensitive to high concentration of miR-30e were PI3K-Akt signaling pathway, pertussis, cytokine-cytokine receptor interaction, MAPK signaling pathway, pathways in cancer, complement and coagulation cascades, proteoglycans in cancer, metabolic pathways, leishmaniasis and p53 signaling pathway. Table 3 contains the top 10 significantly enriched pathways of the DEGs analyzed by KEGG analysis.

Gene Signal Network and pathway Relation Network

We performed pathway relation network analysis to draw a reciprocity network covering 35 significantly changed pathways (Figure 4). Among them, MAPK signaling pathway (degree = 16), apoptosis (degree = 15), pathways in cancer (degree = 15) and cell cycle (degree = 12) showed highest degree, suggesting that these four pathways might play an important role in apoptosis induced by miR-30e treatment. The top 10 significantly changed pathways were listed in Table 4. Based on the obvious regulated GOs and pathways, we selected intersected genes and further constructed mRNAs-GO-networks to screen the key regulatory functions of the identified mRNAs and their target genes, respectively. As shown in Figure 5 and Table 5, the top rated 10 mRNAs including IL-6, Myd88, Fos, Ppap2b, Tlr2, Tlr4, Hk2, Wnt4, C3 and Gstk1. Apart from the gene Tlr4, the other mRNAs were down-regulated by miR-30e treatment in mice.

Discussion

Vascular calcification is a complex biological process associated with aging and degenerative changes [13]. Studies have shown that vascular calcification is an active process that similar to bone formation and it is regulated by multiple factors involving vascular smooth muscle cells, macrophages, endothelial cells, fibroblasts, multiple signal molecules (AKT, KLFS, Smads etc.). Atherosclerosis risk factors include dyslipidemia, hypertension, diabetes, renal failure and this can promote the occurrence and development of arterial calcification [14,15]. The pathogenesis of vascular calcification is currently considered to be related with intravascular bone formation [16]. From the article "miR-30e targets IGF2-regulated osteogenesis in bone marrow-derived mesenchymal stem cells, aortic smooth muscle cells, and ApoE2/2 mice", we known that miR-30e inhibited the osteogenesis in SMCs by targeting IGF2 and suppressed their differentiation into adipogenic or smooth muscle lineage [10].

In this study, gene expression data of 3 SMCs + miR-30e samples and 3 SMCs + ct-miR samples were recovered from the GEO dataset under the accession number GSE65435. The study analyzed 620 DEGs between SMCs + miR-30e samples and SMCs + ct-miR samples, among which 249 genes were up-regulated and 371 were down-regulated. Interestingly, the top 10 DEGs were all down-regulated. So, we speculated that the down-regulated genes were related with osteogenesis. For purpose of better concluded the reciprocity of DEGs, we further analyzed GO analysis and KEGG pathway analysis.

The GO term analysis indicated that DEGs were mainly concluded negative regulation of apoptotic process, cell adhesion, and positive regulation of transcription from RNA polymerase II promoter. It is suggested that miR-30e may promote the process of osteoblast apoptosis, and promote the activation of adipocyte cells, leading to the differentiation of adipocytes in SMCs. Furthermore, enriched KEGG

Table 2. The top 10 mRNAs with high degrees of mRNAs-GO-analysis.

GO ID	GO Name	Diff Gene Counts in GO	Gene Amount in GO	Gene Symbols
GO:0043066	negative regulation of apoptotic process	38	480	|Nr3c1|Itga6|Spp1|Dapk1|Dhcr24|Cyr61|Gata6|Gas1|Cxcr7|Sod2|Foxc2|Atf5|Wnt4|Clu|Sgk1|Angptl4|Il6| Fas|Ier3|Snai2|Pten|Ar|Cth|Aqp1|Serpine1|Mical1|Vnn1|Nes|Cebpb|Timp1|Fgf10|Jak2|Thbs1|Osr1|Btg2| Gas6|Sfrp1|Ivns1abp|
GO:0007155	cell adhesion	38	500	|Cdon|Tgfbi|Pcdh11x|Nrp2|Emb|Tenm3|Mcam|Col6a2|Cdh10|Ptk7|Podxl|Itga6|Pcdh19|Pcdh18|Svep1| Alcam|Spp1|Cd97|Cdh3|Scarf2|Cd34|Emilin2|Postn|Col6a1|Cdh2|Nuak1|Edil3|Adam12|Tnfaip6| Ppap2b|Fbln5|Pdpn|Thbs1|Cyr61|Perp|Pcdh9|Cxcr7|Selp|
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	43	745	|Nfatc4|Tlr4|Cebpb|Itga6|Ablim1|Ankrd1|Zbtb38|Gata2|Il6|Btg2|Id4|Igf2|Ar|Egr1|Runx1|Foxg1|Fgf10| Jag1|Foxf2|Nr4a1|Glis3|Prl2c2|Grem1|Osr1|Lif|Mecom|Fos|Sdpr|Foxc2|Tlr2|Cebpd|Hipk2|Rarb|Il33| Nr3c1|Myd88|Tnip1|Cdon|Gata6|Cyr61|Fgfr2|Il1a|Pbx1|
GO:0006954	inflammatory response	25	214	|S1pr3|Cd14|Cxcl1|Nfkbiz|Lipa|Vnn1|Lxn|Ccl2|Selp|Il1a|Tlr4|Tnip1|Ccl7|Mapkapk2|Il6|Tlr2|C3|Cxcl5| Il8|Thbs1|Myd88|Nos2|Bdkrb1|Mecom|Ptgs2|
GO:0043065	positive regulation of apoptotic process	26	258	|Igfbp3|Bnip3|Dusp6|Uaca|Gata6|Ptgs2|Itga6|Nupr1|Sept4|Dapk1|Map3k5|Nfatc4|Jak2|Cyr61|Tlr4|Fosl1| Lpar1|Ier3|Sfrp1|Nr4a1|Pten|Nox4|Rarb|Ankrd1|Fas|Nos2|
GO:0008285	negative regulation of cell proliferation	28	315	|Jak2|Ptch1|Ptgs2|Sod2|Fgf10|Dhcr24|Cth|Il6|Rarb|Btg2|Fosl1|Rerg|Ereg|Pten|Igfbp3|Adarb1|Il1a|Lif| Serpine2|Cav1|Slc9a3r1|Tlr2|Fgfr2|Sfrp1|Nox4|Il1rl1|Pkp2|Slit3|
GO:0007275	multicellular organismal development	41	954	|Sema3c|Enc1|Ano1|Sfrp1|Tmem2|Nxn|Atoh8|Prrx1|Fzd3|Mgp|Pdpn|Nrp2|Zfp521|Wls|Fhl1|Smoc1| Snai2|Sema3e|Pdgfd|Fzd4|Slc7a5|Ebf1|Foxc2|Jag1|Serpine2|Ereg|Eid2|Olfml3|Pdgfra|Ppap2b|Nes| Gadd45g|Wnt4|Cxcr7|Foxg1|Fyn|Edil3|Sema3a|Pak3|Mecom|Slit3|
GO:0006468	protein phosphorylation	33	611	|Cpne3|Sgk1|Rps6ka6|Nuak1|Trib3|Trib2|Map3k5|Pdk1|Pdgfra|Fgfr2|Gas6|Tgfbr3|Jak2|Hipk2|Pdk4| Mapkapk2|Alpk1|Stk17b|Ksr1|Mlkl|Map4k4|Dapk1|Ptk7|Map3k8|Bmp2k|Prkar2b|Pak3|Mark1|Nek6| Ccne1|Trib1|Fyn|Igfbp3|
GO:0006915	apoptotic process	30	540	|Peg10|Ank2|Slc40a1|Gulp1|Map3k5|Nek6|Dapk1|Perp|Lcn2|Hipk2|Fgfr2|Casp4|Mecom|Nod1|Stk17b| Prune2|Sgk1|Trib3|Chac1|Phlpp1|Gadd45g|Slc9a3r1|Bnip3|Rnf130|Pten|Crip1|Serpina3g|Fas|Nr4a1| Sema3a|
GO:0045766	positive regulation of angiogenesis	15	92	|Cd34|F3|Aqp1|C3ar1|Prl2c2|Chi3l1|Gata2|Grem1|C3|Il1a|Hipk2|Serpine1|Runx1|Thbs1|Gata6|

Table 3. The top 10 mRNAs with high degrees of mRNAs-pathway-analysis.

Pathway ID	Pathway Name	Diff Gene Counts in Pathway	Gene Amount in Pathway	Gene Symbols
4151	PI3K-Akt signaling pathway	25	356	|Pten|Nr4a1|Ccne1|Jak2|Pdgfra|Itga6|Fgfr2|Il4ra|Fgf23|Osmr|Ghr|Tlr2|Fgf10|Il6|Pdgfd|Tlr4|Col6a2| Lpar1|Spp1|Thbs1|Sgk1|Lpar4|Col6a1|Col3a1|Phlpp1|
5133	Pertussis	14	74	|C4bp|Gm5077|Cd14|Il1a|Cxcl5|C3|Serping1|Nos2|Tlr4|Nod1|C1s|Fos|Il6|Myd88|
4060	Cytokine-cytokine receptor interaction	21	266	|Il6|Lifr|Osmr|Il8|Pdgfd|Il4ra|Pdgfra|Il1a|Lif|Ppbp|Il13ra1|Ccl2|Cxcl1|Tnfrsf9|Il18rap|Cxcl5|Ghr| LOC100861978|Fas|Cxcr7|Ccl7|
4010	MAPK signaling pathway	20	259	|Pdgfra|Fgf23|Il1a|Fas|Dusp4|Map3k8|Nfatc4|Gadd45g|Fos|Map3k5|Mapkapk2|Rps6ka6|Dusp6| Mecom|Cacna1c|Nr4a1|Fgf10|Fgfr2|Map4k4|Cd14|
5200	Pathways in cancer	22	326	|Fang|Rarb|Fgf23|Fgf10|Wnt4|Dapk1|Slc2a1|Mecom|Fzd3|Fgfr2|Ptgs2|Ar|Fos|Runx1|Pten|Ccne1| Ptch1|Nos2|Itga6|Il6|Pdgfra|Fzd4|
4610	Complement and coagulation cascades	11	77	|Serpine1|Thbd|C3|F3|C4bp|Serping1|Cfh|Bdkrb1|C1s|C3ar1|Gm5077|
5205	Proteoglycans in cancer	16	230	|Tlr4|Fgf23|Thbs1|Wnt4|Sdc4|Pdk1|Fgf10|Cav1|Tlr2|Fzd4|Igf2|Ptch1|Ank2|Fas|Ank3|Fzd3|
1100	Metabolic pathways	35	1242	|Uprt|Ivd|Pon3|Idi1|Acot1|Gda|Ldhb|Glce|B3galt1|Gcnt2|B3gnt3|Crls1|Ctps|Mtm1|Mboat2|Isyna1| Dhcr24|Eno3|Asah1|Nos2|Alpl|Hk2|Acot2|Dhrs3|Ppap2b|Pcx|Aldh3a1|A4galt|Adh7|Enpp3|Cth|Gcnt4| Sqle|Ptgs2|H6pd|
5140	Leishmaniasis	9	66	|Tlr4|Jak2|Nos2|Il1a|Myd88|Fos|Tlr2|Ptgs2|C3|
4115	p53 signaling pathway	9	69	|Thbs1|Fas|Ccne1|Serpine1|Pten|Gadd45g|

pathways of DEGs including PI3K-Akt signaling pathway, pertussis, cytokine-cytokine receptor interaction. Previous studies showed that PI3K-Akt signaling pathway is a key pathway for osteoclast activation [17,18]. MiR-30e promoted osteoclast activation by active this pathway, and then leaded to osteoblasts reduction. The pertussis toxin-insensitive CCR5 signaling in macrophage pathway was inhibited in the SMCs + miR-30e group and inhibited calcium transport into cells [19]. This pathway also contributed to the reduction of osteogenesis in the SMCs cells. The cytokine-cytokine receptor interaction pathway targets on cells and promotes adipogenic differentiation [20,21].

We also analyzed the mRNAs pathway network with DEGs and list the top degree genes: IL-6, Myd88, Fos, Ppap2b, Tlr2, Tlr4, Hk2, Wnt4, C3 and Gstk1. Among them, IL-6 expression in the highest degree. Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine [22,23]. T cells and macrophages secreted the interleukin 6 and stimulate the immune response. In addition, IL-6 also secreted by the osteoblasts to stimulate osteoclast formation [24]. Smooth muscle cells of many blood vessels also secreted IL-6 as a pro-inflammatory cytokine indicating that miR-30e acts in the regulation of IL-6 [25]. The second

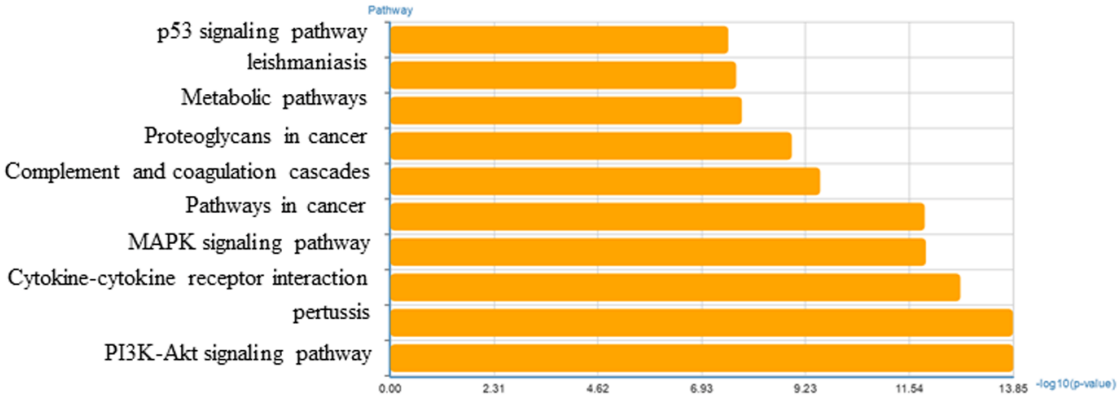

Figure 3. Significantly changed pathways of predicted target gene.

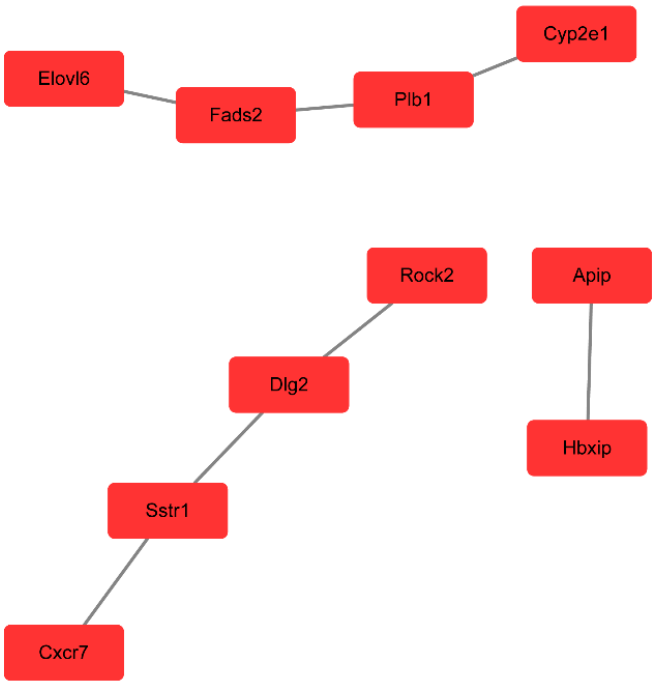

Figure 4. Pathway network (Path-net). Significantly changed pathways were connected in a Path-net to show the interaction network among these pathways.

Table 4. The top 10 mRNAs with high degrees of mRNAs-GO-network.

Pathway ID	Pathway Name	Degree	Pathway Feature
4010	MAPK signaling pathway	16	down/up
4210	Apoptosis	15	down
5200	Pathways in cancer	15	up/down
4510	Focal adhesion	12	up/down
4115	p53 signaling pathway	9	down/up
4060	Cytokine-cytokine receptor interaction	9	down/up
4310	Wnt signaling pathway	8	down
10	Glycolysis / Gluconeogenesis	7	Up/down
4620	Toll-like receptor signaling pathway	6	down/up
4630	Jak-STAT signaling pathway	6	up/down

gene myeloid differentiation primary response gene 88 (MYD88) is a key linker in the Toll-like receptor (TLR) signaling pathway and plays an important role in the transmission of upstream information and disease progression [26]. Toll-like receptors (TLRs) are a class of proteins that play an important role in the innate immune system [27]. From this, we speculate that SMC differentiation is related to the immune system. Ppap2b lets it to regulate vascular and embryonic development by inhibited LPA signaling, which is associated with many human diseases, including cardiovascular disease and cancer, as well as developmental defects [28]. Tlr2 also belong to Toll-like receptor (TLR) signaling pathway. The above results indicated that multiple factors in the body affected the osteogenic differentiation of SMC.

Module analysis of the mRNAs GO network showed that the SMC osteogenesis differentiation was associated with MAPK signaling pathway, apoptosis, and pathways in cancer. The MAPK pathway communicated a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell. MAPK signaling pathway also promoted the differentiation of osteoclasts, and related with calcification in SMCs [29]. Studies have also showed that MAPK pathway is associated

Table 5. The top 10 mRNAs with high degrees of mRNAs-pathway-network.

Gene Symbol	Gene Feature	Biotype	Gene Description	Degree
Il6	down	coding	"Mus musculus interleukin 6 (Il6), mRNA."	20
Myd88	down	coding	"Mus musculus myeloid differentiation primary response gene 88 (Myd88), mRNA."	10.5
Fes	down	coding	"Mus musculus FBJ osteosarcoma oncogene (Fos), mRNA."	7.5
Ppap2b	down	coding	"Mus musculus phosphorous acid phosphatase type 2B (Ppap2b), mRNA."	6
Tlr2	down	coding	"Mus musculus toll-like receptor 2 (Tlr2), mRNA."	4.5
Tlr4	up	coding	"Mus musculus toll-like receptor 4 (Tlr4), mRNA."	4.5
Hk2	down	coding	"Mus musculus hexokinase 2 (Hk2), mRNA."	2
Wnt4	down	coding	"Mus musculus wingless-related MMTV integration site 4 (Wnt4), mRNA."	2
C3	down	coding	"Mus musculus complement component 3 (C3), mRNA."	1
Gstk1	down	coding	"Mus musculus glutathione S-transferase kappa 1 (Gstk1), nuclear gene encoding mitochondrial protein, mRNA."	1

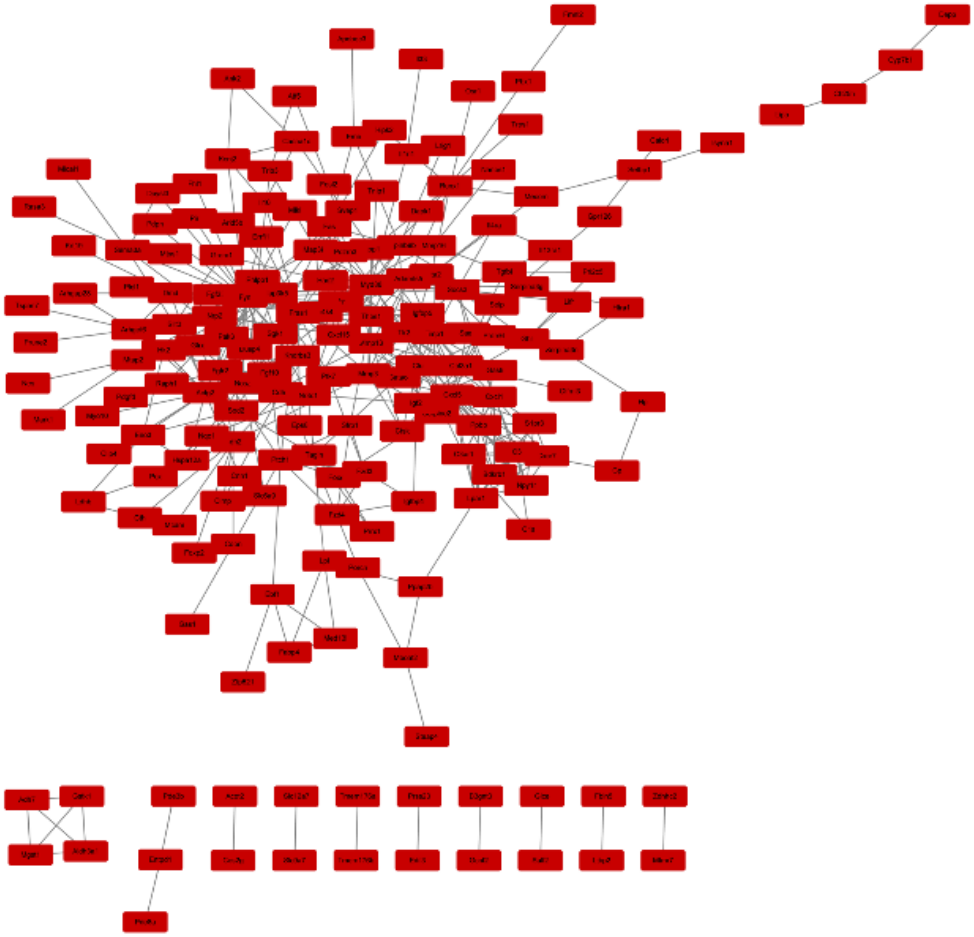

Figure 5. mRNAs gene network. According to the interactions between miRNAs and the intersected target genes, miRNAs-gene-network was constructed to illustrate the key regulatory functions of the identified miRNAs and their target genes.

with cancer [30]. The results showed that calcification in SMCs also related with pathways in cancer. We hypothesized that when a cancer-related pathway is activated, it causes a series of cellular responses, including vascular calcification.

Conclusion

In this study, we provide an integrated bioinformatics analysis of DEGs, which may be included in the progress of differentiation of SMCS. The study showed a serious of important targets for future research into the molecular mechanisms and biomarkers. We hope that these genetic analyzes contribute to the study of vascular calcification.

Availability of data and material

miR-30e targets IGF2-regulated osteogenesis in bone marrow-derived mesenchymal stem cells, aortic smooth muscle cells, and ApoE2/2 mice.

Competing interests

The authors have no conflicts of interest to disclose.

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None.

Authors' contributions

Zhi X., Chen X. and Su J.C. designed this study. Zhi X. analyzed the data. All authors read and approved the final manuscript.

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