Exploring the molecular mechanisms of osteosarcoma by the integrated analysis of mRNAs and miRNA microarrays

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Received December 23, 2015; Accepted August 11, 2017

DOI: 10.3892/ijmm.2018.3594

Abstract. Osteosarcoma (OS) is the most frequently occurring primary bone malignancy with a rapid progression and poor survival. In the present study, in order to examine the molecular mechanisms of OS, we analyzed the microarray of GSE28425. GSE28425 was downloaded from Gene Expression Omnibus, which also included the miRNA expression profile, GSE28423, and the mRNA expression profile, GSE28424. Each of the expression profiles included 19 OS cell lines and 4 normal bones. The differentially expressed genes (DEGs) and differentially expressed miRNAs (DE-miRNAs) were screened using the limma package in Bioconductor. The DEGs associated with tumors were screened and annotated. Subsequently, the potential functions of the DEGs were analyzed by Gene Ontology (GO) and pathway enrichment analyses. Furthermore, the protein-protein interaction (PPI) network was constructed using the STRING database and Cytoscape software. Furthermore, modules of the PPI network were screened using the ClusterOne plugin in Cytoscape. Additionally, the transcription factor (TF)-DEG regulatory network, DE-miRNA-DEG regulatory network and miRNA-function collaborative network were separately constructed to obtain key DEGs and DE-miRNAs. In total, 1,609 DEGs and 149 DE-miRNAs were screened. Upregulated FOS-like antigen 1 (*FOSL1*) also had the function of an oncogene. MAD2 mitotic arrest deficient-like 1 (MAD2L1; degree, 65) and aurora kinase A (AURKA; degree, 64) had higher degrees in the PPI network of the DEGs. In the TF-DEG regulatory network, the TF, signal transducer and activator of transcription 3 (*STAT3*) targeted the most DEGs. Moreover, in the DE-miRNA-DEG regulatory network, downregulated *miR-1* targeted many DEGs and estrogen receptor 1 (*ESR1*) was targeted by several highly expressed miRNAs. Moreover, in the miRNA-function collaborative networks of upregulated miRNAs, *miR-128* targeted myeloid dendritic associated functions. On the whole, our data indicate that *MAD2L1*, *AURKA*, *STAT3*, *ESR1*, *FOSL1*, *miR-1* and *miR-128* may play a role in the development and/or progressio of OS.

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Key words: osteosarcoma, differentially expressed genes, differentially expressed miRNAs, protein-protein interaction network, regulatory network, miRNA-function collaborative network

Introduction

As a high-grade type and mesenchymally-derived bone sarcoma (1), osteosarcoma (OS) is the most prevalent primary bone cancer and the 8th most frequent type of cancer affecting young patients (2). Being characterized by a high malignant degree, rapid progression and a poor survival, OS consists up to 15% of all solid extracranial cancers in patients aged 15-19 years (3,4). Thus, it is necessary to identify biomarkers involved in OS.

DNA repair gene RecQ protein-like 4 (*RECQL4*) is overexpressed in OS, and its overexpression is related to overall genomic instability (5). Human epidermal growth factor receptor 2 (*Her-2/neu*) expression can induce lung metastasis in OS and may be related to gene amplification (6). Overexpressed c-fos (*FOS*) and runt-related transcription factor 2 (*RUNX2*) may play a role in OS; in particular, *RUNX2* expression may serve as a marker of chemotherapy failure in patients with OS (7,8). The cell cycle regulator, CDC5 cell division cycle 5-like (*CDC5L*), is essential for the G2-M transition and may be potential oncogene for the 6p12-p21 amplicon in OS (9). It has been reported that genes with the function of transcription factors (TFs) can also play a role in OS, such as Yin Yang 1 (*YY1*), which is expressed in the early process of osteoblastic transformation and its detection may be used as a promising diagnostic method in human OS (10). In addition, the TF osterix (*Osx*) can suppress the lung migration of OS

tumor cells; thus, the expression of *Osx* may be implicated in the growth and metastasis of OS (11).

There are also many studies which have investigated the direct or indirect effect of microRNAs (miRNAs or miRs) on OS. For example, by targeting matrix metalloprotease 13 (*MMP13*) and B-cell CLL/lymphoma 2 (*Bcl-2*), *miR-143* may be involved in the lung metastasis of human OS cells and may thus be used as a target in cancer therapy (12,13). In addition, downregulated *miR-199a-3p* may function in the growth and proliferation of OS cells; hence, restoring the function of *miR-199a-3p* may contribute to the treatment of OS (14). By mediating reversion-inducing-cysteine-rich protein with kazal motifs (*RECK*), *miR-21* plays an important role in regulating cell invasion and migration in OS and may be a potential therapeutic target (15). By regulating *c-Met* and other genes, *miR-34a* can function as a tumor suppressor gene and suppresses the pulmonary metastasis of OS; thus, it may be a useful gene therapeutic agent (16).

In 2012, Namløs *et al* (17) used global microarray analyses to identify the differentially expressed miRNAs (DE-miRNAs) between OS cell lines and normal bones, and obtained 177 DE-miRNAs. In this study, using the same data by Namløs *et al* (17), we aimed to further screen the differentially expressed genes (DEGs) and DE-miRNAs. The potential functions of the DEGs were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Subsequently, the interaction associations of the proteins encoded by the DEGs were investigated by protein-protein interaction network (PPI) network and modules of PPI network. In addition, the TF-DEG regulatory network, DE-miRNA-DEG regulatory network and miRNA-function collaborative network were separately constructed to obtain key DEGs and DE-miRNAs.

Data collection methods and analysis

Microarray data. The microarray of GSE28425 deposited by Namløs *et al* (17) was downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), which included the miRNA expression profile, GSE28423, and the mRNA expression profile, GSE28424. Each of the expression profiles included a collection of 19 OS cell lines and 4 normal bones. GSE28423 was based on the platform of GPL8227 Agilent-019118 Human miRNA Microarray 2.0 G4470B (Agilent Technologies Inc., Santa Clara, CA, USA). GSE28424 was based on the platform of GPL13376 Illumina HumanWG-6 v2.0 expression beadchip (Illumina, San Diego, CA, USA). The OS samples were from a panel collected within EuroBoNeT and from the Norwegian Radium Hospital. Meanwhile, normal bones were from Capital Biosciences or from amputations of cancer patients at the University College London and Norwegian Radium Hospital.

Screening of DEGs and DE-miRNAs. After GSE28425 was downloaded, the microarray data was pre-processed using the limma package (18) in Bioconductor (http://www.bioconductor.org/packages/release/bioc/html/limma.html). In brief, the pre-processing process included Background Correction, Quantile Normalization and Probe Summarization. The limma (linear models for microarray data) package (18) was used to analyze the DEGs and DE-miRNAs between the OS cell lines and normal bones. The FDR (that is, adjusted p-value) <0.05 and |log2fold-change (FC)| >1 were used as the cut-off criteria. Screening the tumor suppressor (TS) gene (http://bioinfo.mc.vanderbilt.edu/TSGene/download.cgi) (19) and tumor-associated gene (TAG) (http://www.binfo.ncku.edu.tw/TAG/GeneDoc.php) (20) databases, the DEGs associated with tumors were screened and annotated.

Functional and pathway enrichment analysis. GO provides controlled and structured vocabularies which model biological process (BP), cell components (CC) and molecular function (MF) (21). KEGG is a database containing 16 main databases, roughly divided into systems information, chemical information and genomic information (22). GO functional enrichment analyses, which involved the BP, MF and CC categories, as well as KEGG pathway enrichment analyses were performed for the DEGs and the DE-miRNAs. A p-value <0.05 was used as the cut-off criterion.

PPI network and module construction. The interaction associations of the proteins encoded by the DEGs were searched using STRING online software (http://string-db.org; v9.05) (23), and the combined score of >0.7 was used as the cut-off criterion. The PPI network was visualized using Cytoscape software (http://www.cytoscape.org) (24). Modules of the PPI network were screened using the ClusterOne plugin (25) in Cytoscape, and the significant p-value of the modules were set to 1.1E-6.

TF-DEG regulatory network construction. Human TF-gene regulatory pairs were downloaded from the UCSC database (http://genome.ucsc.edu/) (26). The DEGs which can also function as TFs and their target genes were then identified. Moreover, Cytoscape software (24) was used to visualize the the TF-DEG regulatory network.

DE-miRNA-DEG regulatory network construction. By comparing the experimental validated miRNA-mRNA pairs in the miRecords (http://www.mirecords.umn.edu) (27) and mirWalk (http://mirwalk.uni-hd.de/) (28) databases, pairs of DE-miRNAs from the miRNA expression profile, GSE28423, and DEGs from the mRNA expression profile, GSE28424, were obtained. The DE-miRNA-DEG pairs should appear in either miRecords database or mirWalk database.

miRNA-function collaborative network construction. According to the functional enrichment results of the DE-miRNAs, the DE-miRNAs which targeted the genes involved in one BP term were identified. Subsequently, miRNA-function collaborative network was constructed. A p-value <0.01 was used as the cut-off criterion.

Results

DEGs analysis. Compared with normal bones, there were 1,609 DEGs (including 774 upregulated and 835 downregulated mRNAs) and 149 DE-miRNAs (including 76 upregulated and 73 downregulated miRNAs) screened in the OS cell lines. The DEGs associated with tumors were annotated and are listed in Table I. Importantly, upregulated FOS-like antigen 1 (*FOSL1*) also had the function of an oncogene.

Table I. The DEGs associated with tumors.

Category	Oncogene	TSG	TAG
UP	*CDC5L*, *FOSL1*, *HMMR*, *AURKA*, *MLF1*, *CDK4*, *MET*, *TRIO*, *NRAS*, *HOXA10*, *WHSC1*, *PIK3CA*	*S100A2*, *TUSC3*, *PAWR*, *LZTS1*, *YAP1*, *GADD45GIP1*, *PTPRG*, *RND3*, *DFNA5*, *HOXB13*, *BAI2*, *ZDHHC2*, *NF2*, *BCL10*, *FANCG*, *AMH*, *RCN2*, *HLTF*, *NME1*, *REV3L*, *DAPK3*, *FH*, *MEN1*, *HECA*, *TRIM3*, *SCRIB*, *BRMS1*, *EXTL3*, *SMARCB1*, *PCGF2*	*TFAP2A*, *BUB1*, *NKX3-1*, *DNMT3B*, *PMS1*, *SHC1*, *YEATS4*, *FADD*, *C1QBP*
DOWN	*FGF20*, *LYN*, *BCL6*, *TAL1*, *ESR1*, *WISP2*, *LMO2*, *LCN2*, *LYL1*	*HSD17B7*, *PRODH*, *MAL*, *DUSP22*, *TSC22D1*, *COL4A3*, *BAI3*, *BNIP3L*, *PER1*, *PAEP*, *RASSF4*, *FOXC1*, *EXTL1*, *ARHGAP20*, *CMTM5*, *NGFR*, *TXNIP*, *NOTCH1*, *MRVI1*, *MTSS1*, *MTUS1*, *PPAP2A*, *TCF4*, *ST5*, *PYHIN1*, *PRKCD*, *TGFBR3*, *CBFA2T3*, *MT1G*, *TSPAN32*, *RASSF2*, *CEBPA*, *LTF*, *RARRES1*, *MAP4K1*, *BTG2*, *PLA2G2A*, *ZBTB16*, *SYK*, *GPX3*, *PYCARD*, *H19*, *PTPN6*, *C2orf40*	*TAL2*, *WISP3*, *STAT3*, *CBLB*, *NR4A2*, *LYST*, *RGS2*, *FES*, *MGP*

TSG, tumor suppressor gene; TAG, tumor-associated gene.

Functional and pathway enrichment analysis. The top 5 enriched GO functions in the BP, CC and MF categories separately for the upregulated and downregulated genes are listed in Table II. For the upregulated genes, the enriched functions included cell cycle (p=0), intracellular membrane-bounded organelle (p=0) and catalytic activity (p=3.05E-10). For the downregulated genes, the enriched functions included cell activation (p=0), extracellular region (p=0) and carbohydrate derivative binding (p=1.55E-08).

The top 10 enriched KEGG pathways separately for the upregulated and downregulated genes are also listed in Table II. For the upregulated genes, the enriched pathways included metabolic pathways (p=1.14E-06), steroid biosynthesis (p=2.02E-05) and spliceosome (p=0.003765459). For the downregulated genes, the enriched pathways included cytokine-cytokine receptor interaction (p=3.76E-06) and osteoclast differentiation (p=7.87E-06).

PPI network and module analysis. The PPI network of the DEGs had 844 nodes and 3,400 interactions. In particular, MAD2 mitotic arrest deficient-like 1 (MAD2L1, degree, 65), cyclin B1 (CCNB1, degree, 65) and aurora kinase A (AURKA, degree, 64) had high degrees in the PPI network. In addition, 3 modules (module 1, module 2 and module 3) of the PPI network were screened (Fig. 1). In module 1, TAO kinase 1 (TAOK1) was the only downregulated gene. The enriched KEGG pathways for the DEGs in module 1 included oocyte meiosis (p=2.04E-08), cell cycle (p=4.16E-08) and progesterone-mediated oocyte maturation (p=0.000112373) (Table III). In module 2, guanine nucleotide binding protein, α inhibiting 1 (GNAI1) and regulator of G-protein signaling 20 (RGS20) were downregulated. The enriched KEGG pathways for the DEGs in module 2 included chemokine signaling pathway (p=0) and cytokine-cytokine receptor interaction (p=9.77E-15) (Table III). Furthermore, the DEGs involved in module 3 were all upregulated genes. The enriched KEGG pathways for the DEGs in module 3 included ribosome (p=1.26E-12) and protein processing in endoplasmic reticulum (p=0.043084724) (Table III).

TF-DEG regulatory network analysis. The TF-DEG regulatory network had 311 interactions (involving 10 transcription factors and 285 DEGs) (Fig. 2). Importantly, the TFs, signal transducer and activator of transcription 3 (*STAT3*, degree, 158) and forkhead box A1 (*FOXA1*, degree, 106) targeted the most DEGs.

DE-miRNA-DEG regulatory network analysis. The DE-miRNA-DEG regulatory network involved 23 upregulated miRNAs and 64 downregulated miRNAs (Fig. 3). In the DE-miRNA-DEG regulatory network, downregulated *miR-1* targeted and activated many DEGs. Moreover, downregulated estrogen receptor 1 (*ESR1*) was targeted by several high-expressed miRNAs, including miR-221, miR-20b and miR-18a. The enriched GO functions for the upregulated and downregulated miRNAs are listed in Table IV. For the upregulated miRNAs, the enriched functions included positive regulation of retinoic acid receptor signaling pathway (p=0.000211583) and type 1 metabotropic glutamate receptor binding (p=0.000150457). For the downregulated miRNAs, the enriched functions included response to inactivity (p=0.001989302) and potassium ion binding (p=0.006643278).

miRNA-function collaborative network analysis. The miRNA-function collaborative networks of upregulated (Fig. 4) and downregulated (Fig. 5) miRNAs were constructed, respectively. In the miRNA-function collaborative networks of upregulated miRNAs, myeloid dendritic associated functions were targeted by miR-128 and miR-125a-5p.

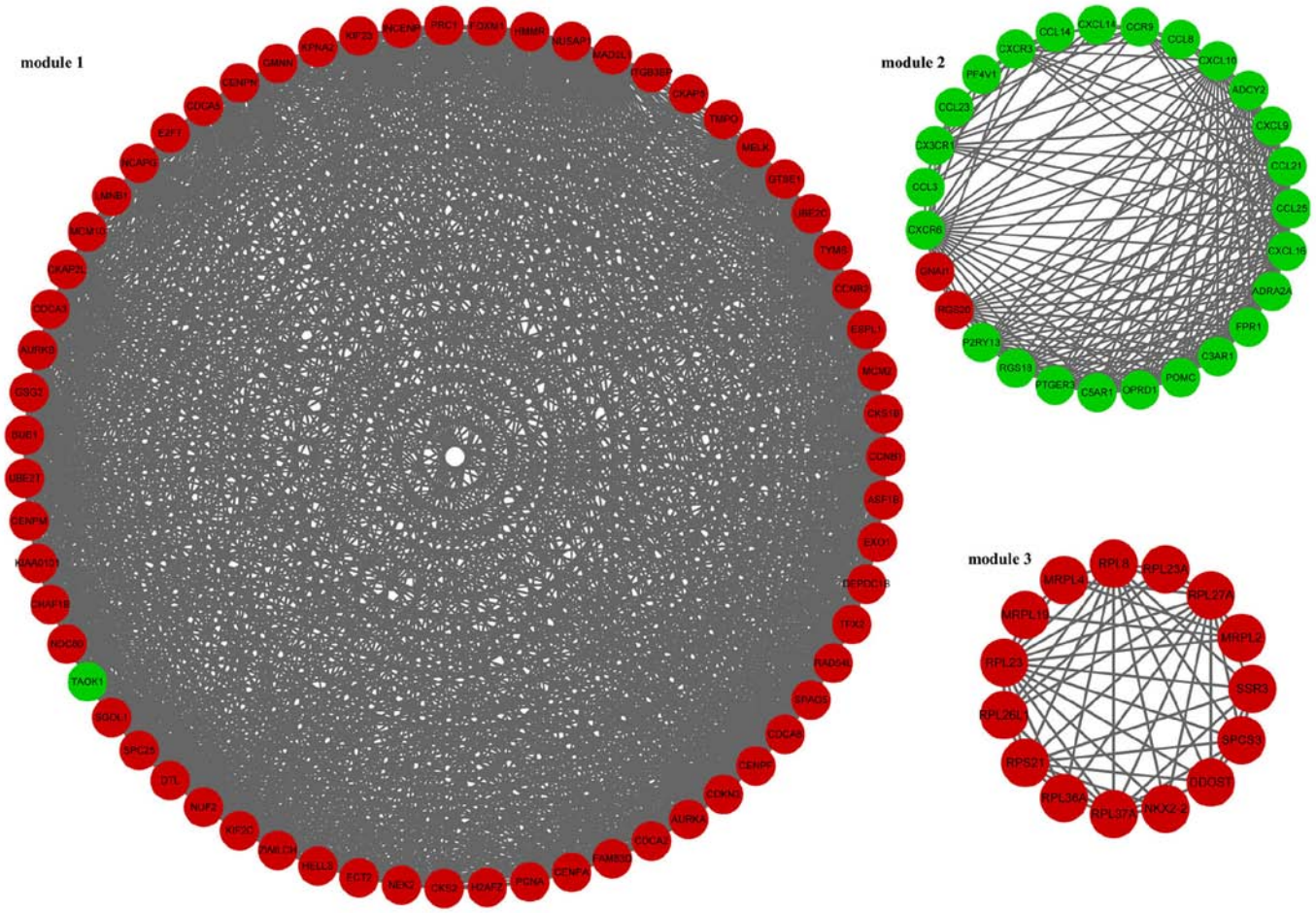

Figure 1. The three modules (module 1, module 2 and module 3) screened from the protein-protein interaction (PPI) network. The red circle nodes represent the upregulated genes, while the green circle nodes represent the downregulated genes.

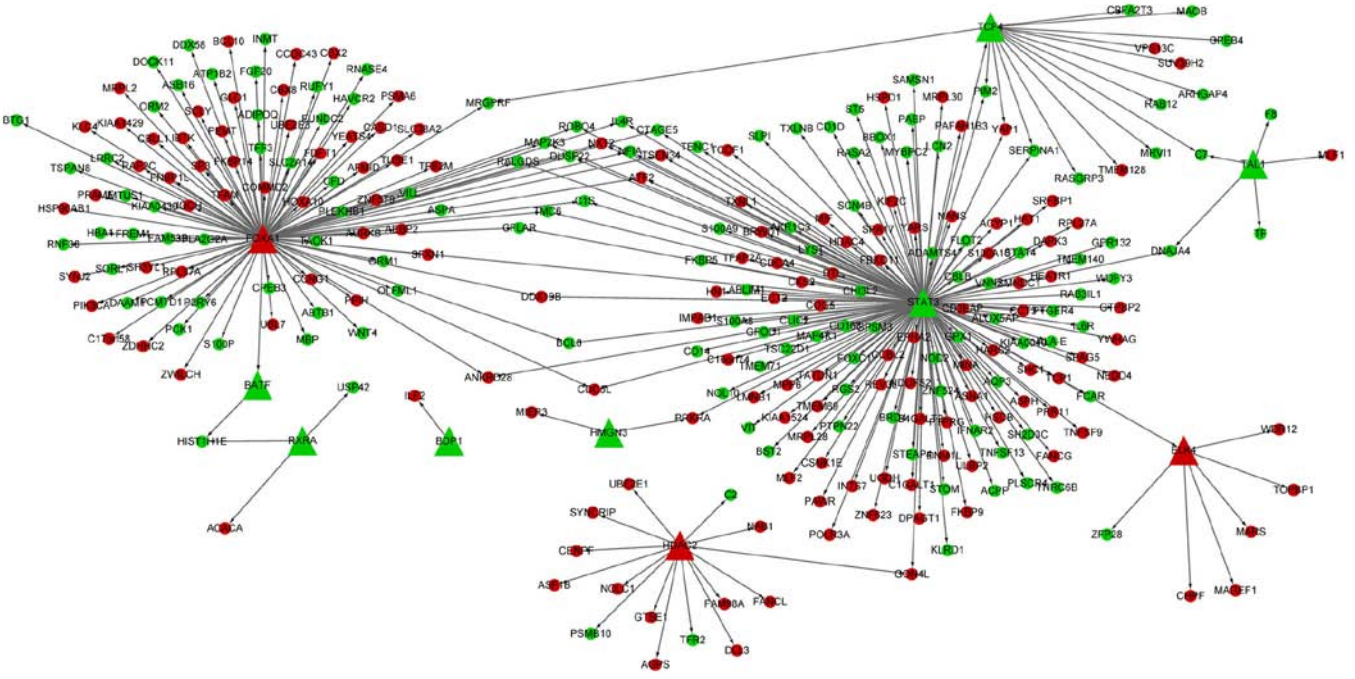

Figure 2. The TF-differentially expressed genes (DEG) regulatory network. The red nodes represent the upregulated genes, while the green nodes represent the downregulated genes. In addition, the triangle nodes stand for transcription factors, and circles nodes stand for their targeted genes.

Table II. The top 5 enriched GO functions in BP, CC and MF categories, as well as the top 10 enriched KEGG pathways separately for the upregulated and downregulated genes.

Category	Term	Description	Gene no.	Gene symbol	p-value
UP_BP	GO:0007049	Cell cycle	133	*KPNA2*, *UBE2C*	0
	GO:0000278	Mitotic cell cycle	90	*CDCA3*, *E2F7*	2.22E-16
	GO:0022402	Cell cycle process	108	*FAM83D*, *SPC25*	1.22E-15
	GO:0051301	Cell division	62	*UBE2C*, *CDCA3*	3.86E-14
	GO:0048285	Organelle fission	52	*FAM83D*, *SPC25*	7.72E-14
UP_CC	GO:0005622	Intracellular	611	*TFAP2A*, *CBS*	0
	GO:0031981	Nuclear lumen	161	*CBS*, *KPNA2*	0
	GO:0043231	Intracellular membrane-bounded organelle	509	*KPNA2*, *JPH3*	0
	GO:0044422	Organelle part	384	*SHROOM3*, *UBE2C*	0
	GO:0044424	Intracellular part	607	*FOXD1*, *UBE2C*	0
UP_MF	GO:0003824	Catalytic activity	300	*PSAT1*, *UBE2C*	3.05E-10
	GO:0016740	Transferase activity	123	*PSAT1*, *CCNB1*	3.95E-09
	GO:0005515	Protein binding	382	*TFAP2A*, *CBS*	1.13E-07
	GO:0032549	Ribonucleoside binding	115	*UBE2C*, *KIF2C*	3.57E-06
	GO:0035639	Purine ribonucleoside triphosphate binding	114	*SEPT3*, *PTK7*	4.27E-06
DOWN_BP	GO:0001775	Cell activation	104	*GRAP2*, *IL12RB1*	0
	GO:0001816	Cytokine production	73	*STAT5B*, *LIPA*	0
	GO:0002376	Immune system process	252	*FGF20*, *FCGR3A*	0
	GO:0002682	Regulation of immune system process	153	*BLK*, *CD200R1*	0
	GO:0002684	Positive regulation of immune system process	99	*FCGR3A*, *GRAP2*	0
DOWN_CC	GO:0005576	Extracellular region	191	*FGF20*, *FCGR3A*	0
	GO:0005615	Extracellular space	98	*CCL25*, *APOC2*	0
	GO:0005886	Plasma membrane	316	*IL12RB1*, *BLK*	0
	GO:0044421	Extracellular region part	120	*IL12RB1*, *BLK*	0
	GO:0044459	Plasma membrane part	174	*OPRD1*, *MAL*	0
DOWN_MF	GO:0097367	Carbohydrate derivative binding	29	*FGF7*, *TLR2*	1.55E-08
	GO:0005515	Protein binding	417	*FGF20*, *HMGN3*	3.54E-08
	GO:0046983	Protein dimerization activity	80	*ADD2*, *APOC2*	5.30E-08
	GO:0008307	Structural constituent of muscle	13	*DMD*, *MYL4*	9.25E-08
	GO:0042803	Protein homodimerization activity	55	*MZF1*, *ADD1*	1.31E-07
UP_KEGG	01100	Metabolic pathways	89	*CBS*, *PSAT1*	1.14E-06
	00100	Steroid biosynthesis	7	*DHCR24*, *SQLE*	2.02E-05
	03040	Spliceosome	14	*CDC5L*, *SMNDC1*	0.003765459
	03008	Ribosome biogenesis in eukaryotes	10	*NXT2*, *NMD3*	0.005664746
	00270	Cysteine and methionine metabolism	6	*CBS*, *DNMT3B*	0.007673661
	00510	N-Glycan biosynthesis	7	*TUSC3*, *ALG10B*	0.009627025
	00970	Aminoacyl-tRNA biosynthesis	8	*MARS*, *YARS*	0.011699718
	00620	Pyruvate metabolism	6	*ME1*, *ACAT2*	0.012824724
	00290	Valine, leucine and isoleucine biosynthesis	3	*BCAT1*, *VARS*, *LARS*	0.014647016
	01040	Biosynthesis of unsaturated fatty acids	4	*PTPLA*, *ELOVL5*, *PTPLB*, *SCD*	0.017934809
DOWN_KEGG	05150	*Staphylococcus aureus* infection	21	*FCAR*, *C3AR1*	2.29E-12
	04640	Hematopoietic cell lineage	21	*IL4R*, *CR1*	4.15E-08
	04145	Phagosome	27	*TLR2*, *NOX1*	4.14E-07
	05140	Leishmaniasis	17	*CR1*, *IFNGR1*	9.79E-07
	04060	Cytokine-cytokine receptor interaction	36	*CCL25*, *TNFSF8*	3.76E-06

Table II. Continued.

Category	Term	Description	Gene no.	Gene symbol	p-value
	04380	Osteoclast differentiation	22	*LILRA6*, *NOX1*	7.87E-06
	04650	Natural killer cell mediated cytotoxicity	22	*IFNGR1*, *NFATC3*	2.14E-05
	04514	Cell adhesion molecules (CAMs)	21	*MAG*, *F11R*	4.77E-05
	05310	Asthma	9	*MS4A2*, *EPX*	4.91E-05
	05416	Viral myocarditis	14	*DMD*, *SGCA*	6.63E-05

GO, Gene Ontology; BP, biological process; CC, cell components; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

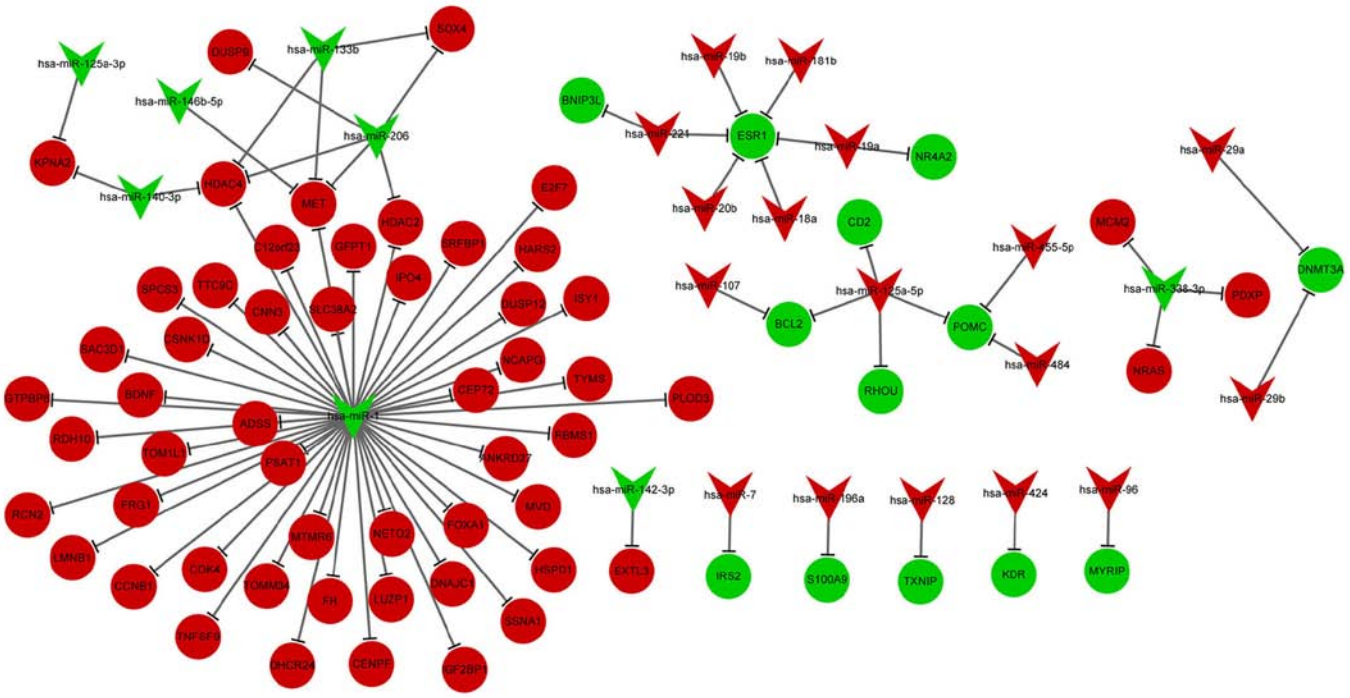

Figure 3. The DE-miRNA-DEG regulatory network. The red nodes represent the upregulated DEGs, while the green nodes represent the downregulated DEGs. In addition, the nodes in inverse triangle stand for miRNAs, and circles nodes stand for their targeted genes. DEGs: differentially expressed genes.

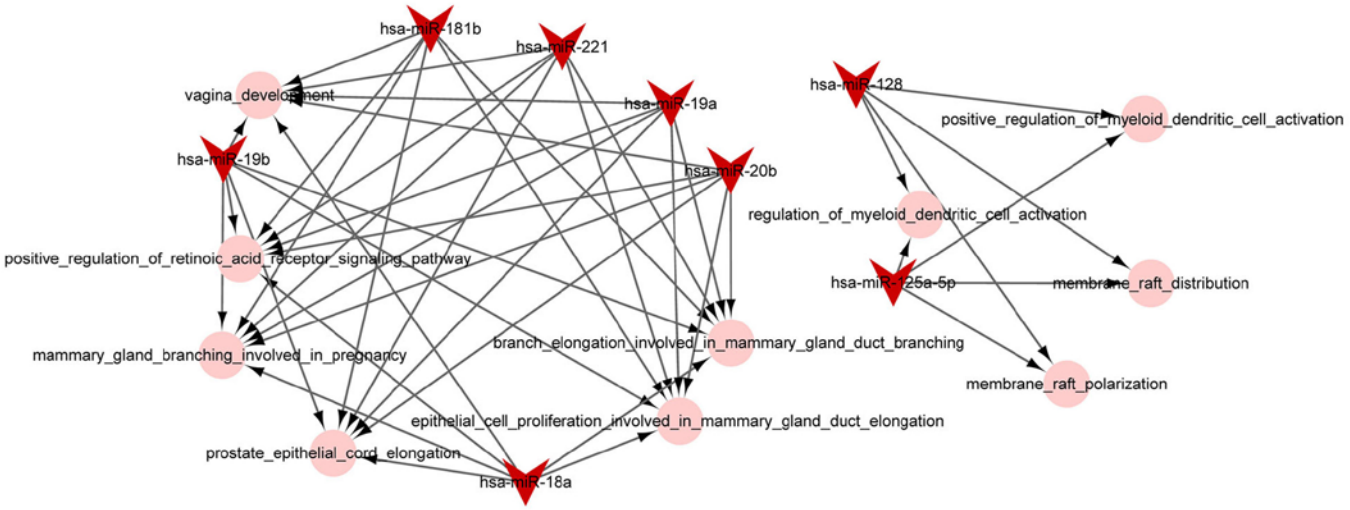

Figure 4. The miRNA-function collaborative network of upregulated miRNAs. The pink circle nodes represent the biological process terms of Gene Ontology.

Table III. The enriched KEGG pathways for the DEGs in module 1, module 2 and module 3 of the PPI network.

	Term	Description	Gene no.	Gene symbol	p-value
Module 1	04114	Oocyte meiosis	7	*AURKA, SGOL1*	2.04E-08
	04110	Cell cycle	7	*PCNA, MCM2*	4.16E-08
	04914	Progesterone-mediated oocyte maturation	4	*CCNB2, BUB1, MAD2L1, CCNB1*	0.000112373
	04115	p53 signaling pathway	3	*CCNB2, CCNB1,GTSE1*	0.001071355
	03430	Mismatch repair	2	*PCNA, EXO1*	0.002163033
	03030	DNA replication	2	*PCNA, MCM2*	0.00526065
Module 2	04062	Chemokine signaling pathway	17	*ADCY2, CX3CR1*	0
	04060	Cytokine-cytokine receptor interaction	15	*CX3CR1, CXCR6*	9.77E-15
	04080	Neuroactive ligand-receptor interaction	7	*OPRD1, P2RY13*	9.93E-05
	05150	Staphylococcus aureus infection	3	*C3AR1, FPR1,C5AR1*	0.001547235
	04916	Melanogenesis	3	*ADCY2, POMC, GNAI1*	0.008639105
	04620	Toll-like receptor signaling pathway	3	*CCL3, CXCL9, CXCL10*	0.008876016
	04672	Intestinal immune network for IgA production	2	*CCL25, CCR9*	0.017427247
	04610	Complement and coagulation cascades	2	*C3AR1, C5AR1*	0.034329309
	04971	Gastric acid secretion	2	*ADCY2, GNAI1*	0.039017822
Module 3	03010	Ribosome	8	*RPL27A, RPL37A*	1.26E-12
	04141	Protein processing in endoplasmic reticulum	2	*DDOST, SSR3*	0.043084724

KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; PPI, protein-protein interaction.

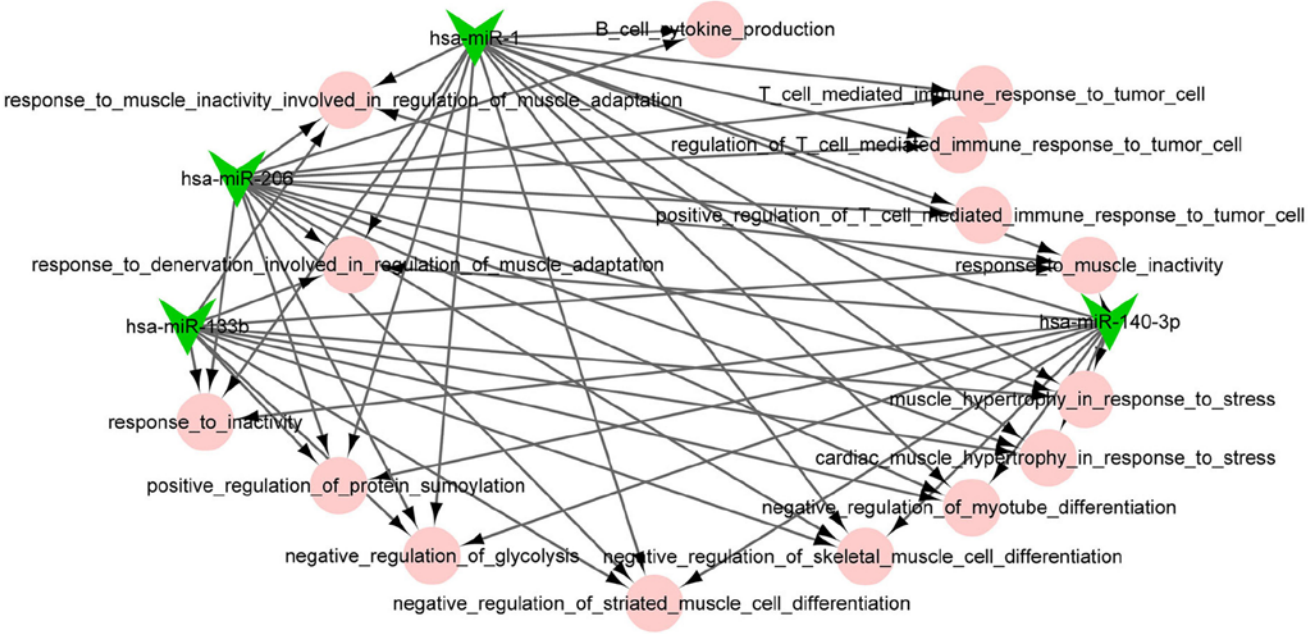

Figure 5. The miRNA-function collaborative network of downregulated miRNAs. The pink circle nodes represent the biological process terms of Gene Ontology.

Discussion

In this study, we screened 1,609 DEGs (including 774 upregulated and 835 downregulated mRNAs) and 149 DE-miRNAs (including 76 upregulated and 73 downregulated miRNAs) in the OS cell lines compared with normal bones. Importantly, upregulated *FOSL1* also had the function of an oncogene. MAD2L1 (degree, 65) and AURKA (degree, 64) had higher degrees in the PPI network of the DEGs. In the DE-miRNA-DEG regulatory network, downregulated *miR-1* targeted many DEGs and *ESR1* were targeted by several highly expressed miRNAs. Moreover, in the miRNA-function collaborative networks of upregulated miRNAs, *miR-128* targeted myeloid dendritic associated functions.

Table IV. The enriched GO functions for the upregulated and downregulated miRNAs involved in the DE-miRNA-DEG regulatory network.

Category	Term	Description	miRNA no.	miRNA symbol	p-value
UP_BP	0048386	Positive regulation of retinoic acid Receptor signaling pathway	6	*miR-221*, *miR-18a*	0.000211583
	0060523	Prostate epithelial cord elongation	6	*miR-20b*, *miR-18a*	0.001058491
	0060745	Mammary gland branching involved in pregnancy	6	*miR-221*, *miR-20b*	0.001196154
	0001766	Membrane raft polarization	2	*miR-125a-5p*, *miR-128*	0.002429205
	0030885	Regulation of myeloid dendritic cell activation	2	*miR-125a-5p*, *miR-128*	0.002429205
	0030887	Positive regulation of myeloid dendritic cell activation	2	*miR-125a-5p*, *miR-128*	0.002429205
UP_MF	0031798	Type 1 metabotropic glutamate receptor binding	6	*miR-221*, *miR-20b*	0.000150457
	0030235	Nitric-oxide synthase regulator activity	6	*miR-19b*, *miR-20b*	0.000413057
	0035256	G-protein coupled glutamate receptor binding	6	*miR-19b*, *miR-18a*	0.000413057
	0030284	Estrogen receptor activity	6	*miR-19a*, *miR-18a*	0.002145221
	0034056	Estrogen response element binding	6	*miR-19b*, *miR-19a*	0.003216968
	0031779	Melanocortin receptor binding	3	*miR-455-5p*, *miR-125a-5p*, *miR-484*	0.009585627
	0031781	Type 3 melanocortin receptor binding	3	*miR-455-5p*, *miR-484*, *miR-125a-5p*	0.009585627
DOWN_BP	0014854	Response to inactivity	4	*miR-133b*, *miR-206*	0.001989302
	0014870	Response to muscle inactivity	4	*miR-1*, *miR-133b*	0.001989302
	0014877	Response to muscle inactivity involved in regulation of muscle adaptation	4	*miR-206*, *miR-1*	0.001989302
	0014894	Response to denervation involved in regulation of muscle adaptation	4	*miR-1*, *miR-133b*	0.001989302
	0002368	B cell cytokine production	2	*miR-206*, *miR-1*	0.002474699
	0002424	T cell mediated immune response to tumor cell	2	*miR-1*, *miR-206*	0.002474699
DOWN_ MF	0005008	Hepatocyte growth factor-activated receptor activity	4	*miR-133b*, *miR-206*	0.001415624
	0030955	Potassium ion binding	4	*miR-206*, *miR-140-3p*	0.006643278
	0031420	Alkali metal ion binding	4	*miR-133b*, *miR-1*	0.007459611
	0003688	DNA replication origin binding	2	*miR-206*, *miR-1*	0.014287542
	0031078	Histone deacetylase activity (H3-K14 specific)	4	*miR-206*, *miR-140-3p*	0.016613831
	0032041	NAD-dependent histone deacetylase activity (H3-K14 specific)	4	*miR-1*, *miR-206*	0.016613831

DEG, differentially expressed gene; BP, biological process; MF, molecular function.

In the PPI network of the DEGs, MAD2L1 and AURKA were with high degrees. The overexpression of Mad2 can induce early dyscrasia, lung metastasis and poor survival in OS (29). The knockdown of *Mad2* leads to OS cell death through apoptosis associated with *Rad21* cleavage; thus, *Mad2* may serve as a target for cancer therapy (30). *AURKA* can promote cell cycle and suppress cell apoptosis, and the inhibition of *AURKA* by specific short hairpin RNA (shRNA) may

be a promising therapeutic strategy of OS (31). Furthermore, in the TF-DEG regulatory network, the TF, *STAT3*, targeted the most DEGs. By binding to the promoter region of *miR-125b* and acting as a transactivator, *STAT3* regulates *miR-125b* which serves as a potential target in the therapy of OS (32). The overexpression of phosphorylated-STAT3 in OS cells is implicated in poor prognosis and may function as a prognostic indicator and therapeutic target for OS (33,34). These data suggest that *MAD2L1*, *AURKA* and *STAT3* may be closely associated with OS.

Some other molecules have also been involved in OS. The deregulation of *miR-1* and *miR-133b* may correlate with cell cycle and cell proliferation of OS by mediating c-met (MET) protein expression (35). Through directly regulating PTEN/AKT signaling, miR-128 functions in the proliferation of human OS cells (36). The hypermethylation of p14ARF and ESR1 separately correlates with the absence of metastases at diagnoses and poor survival, therefore, p14ARF and ESR1 hypermethylation may be used as prognostic indicators for in OS (37). In 143B OS cells, phosphorylated and activated *c-Jun* and *Fra-1* (also known as *FOSL1*) can induce *MMP1* gene expression which may be a target for invasive and pulmonary metastases of OS, therefore, phosphorylated *c-Jun* and *Fra-1* may affect invasion of OS through mediating *MMP1* (38).

In conclusion, this study identified key genes or miRNAs involved in OS. We screened 1,609 DEGs and 149 DE-miRNAs in the OS cell lines compared with normal bones. Besides, some molecules may correlate with OS, such as *MAD2L1*, *AURKA*, *STAT3*, *ESR1*, *FOSL1*, *miR-1* and *miR-128*. However, experimental researches are still necessary to validate the functions of these molecules in OS.

Competing interests

The authors declare that they have no competing interests.

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