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Original Article

Identification of a novel miRNA-target gene regulatory network in osteosarcoma by integrating transcriptome analysis

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Abstract: Osteosarcoma remains a leading cause of cancer death in children and young adolescents. Although the introduction of multiagent chemotherapy, survival rates have not improved in two decades. Therefore, it is urgently needed to know the details regarding molecular etiology to driving therapeutic inroads for this disease. In this study we performed an integrated analysis of miRNA and mRNA expression data to explore the dysregulation of miRNA and miRNA-target gene regulatory network underlying OS. 59 differentially expressed miRNAs were identified, with 28 up-regulated and 31 down-regulated miRNAs by integrating OS miRNA expression data sets available. Using miRWalk databases prediction, we performed an anticorrelated analysis of miRNA and genes expression identified by a integrated analysis of gene expression data to identify 109 differently expressed miRNA target genes. A novel miRNA-target gene regulatory network was constructed with the miRNA-target gene pairs. miR-19b-3p, miR-20a-5p, miR-124-3p and their common target CCND2, the nodal points of regulatory network, may play important roles in OS. Bioinformatics analysis of biological functions and pathways demonstrated that target genes of miRNAs are highly correlated with carcinogenesis. Our findings may help to understand the molecular mechanisms of OS and identify targets of effective targeted therapies for OS.

Keywords: Integrated analysis, miRNA expression data, osteosarcoma, miRNA target genes

Introduction

Osteosarcoma (OS) is the most common primary bone malignancy in children and young adolescents characterized by malignant osteoid production and osteoblastic differentiation. After the introduction of multiagent chemotherapy in the 1980s, the 5-year survival rate has increased to approximate 60%-65% for patients without evidence of metastasis [1]. However, for patients with recurrent or metastatic OS, the prognosis is still very poor [2]. Therefore, it is urgently needed to identify the details regarding tumor progression and to discover new insights into novel therapy strategies for this disease.

MicroRNA (miRNA) are small (~22 nucleotides) non-coding RNAs, which negatively regulates gene expression by binding to the 3'-untranslated region (3'-UTR) of their target mRNA [3]. Thus, over-expression of miRNAs usually gives rise to the deceased expression of target genes. Amounts of evidence show that miRNA are deregulated in various types of cancer and play crucial roles in tumor formation and development [4, 5]. miRNAs is still considered to be applied in diagnosis and prognosis as well as eventual therapy of malignant neoplasm [6].

Complex genomic aberrations and highly variable patterns of gene expression were detected in conventional OS [7]. With advances in molecular biology, emerging evidence using microarray-based approaches shows that miRNAs were deregulated in human OS compared to bone, osteoblasts and mesenchymal stem cells [8-11]. In addition, some studies identified their important role in the development of OS. miR-21 has been indicated to induce invasion and migration of the OS cell line, MG-63, by negatively regulating RECK, a tumor suppressor

Table 1. List of differentially expressed miRNAs

miRNA	*P*-value	Effective_size
Up-regulated miRNAs		
hsa-miR-9-3p	4.33E-09	0.852555909
hsa-miR-15a-3p	3.86E-06	0.709492785
hsa-miR-518b	7.81E-06	0.850142061
hsa-miR-106b-3p	1.82E-05	0.331329545
hsa-miR-149-5p	2.20E-05	0.929734799
hsa-miR-646	4.10E-05	0.85623655
hsa-miR-137	7.91E-05	0.606474533
hsa-miR-182-5p	2.03E-04	0.353731074
hsa-miR-323a-3p	4.28E-04	0.724130102
hsa-miR-624-5p	9.64E-04	0.338806539
hsa-miR-657	1.02E-03	0.26330906
hsa-miR-769-5p	1.11E-03	0.948959053
hsa-miR-20a-5p	1.60E-03	0.594581368
hsa-miR-181a-2-3p	1.82E-03	0.72873165
hsa-miR-608	2.04E-03	0.836196694
hsa-miR-29b-3p	2.61E-03	0.104500708
hsa-miR-153-3p	2.91E-03	0.520093828
hsa-miR-758-3p	3.29E-03	0.801531307
hsa-miR-17-3p	3.55E-03	0.693026831
hsa-miR-100-3p	4.03E-03	0.165013099
hsa-miR-19b-3p	4.53E-03	0.540592183
hsa-miR-650	5.46E-03	0.180498019
hsa-miR-124-3p	5.68E-03	0.566669559
hsa-miR-17-5p	7.50E-03	0.523690599
hsa-miR-106a-3p	8.43E-03	0.207385799
hsa-miR-16-2-3p	8.55E-03	0.432417158
hsa-miR-148b-3p	9.14E-03	0.622875552
hsa-miR-135b-5p	9.42E-03	0.15981984
Down-regulated miRNAs		
hsa-miR-223-3p	3.59E-13	-1.845850571
hsa-miR-126-5p	8.43E-13	-1.053842174
hsa-miR-126-3p	1.36E-12	-1.967999713
hsa-miR-610	4.86E-10	-1.534542956
hsa-miR-671-5p	5.33E-10	-0.97111795
hsa-miR-195-5p	3.09E-08	-1.268764741
hsa-miR-638	1.36E-07	-1.024613101
hsa-miR-142-5p	3.07E-07	-1.834983092
hsa-miR-451a	7.62E-07	-1.232024612
hsa-miR-663a	1.12E-05	-1.234272536
hsa-miR-144-5p	4.66E-05	-1.088489066
hsa-miR-486-5p	4.72E-05	-1.116914298
hsa-miR-623	6.04E-05	-0.986359598
hsa-miR-16-5p	7.02E-05	-3.67194014
hsa-miR-572	8.81E-05	-1.401081994
hsa-miR-139-5p	2.11E-04	-1.170260462
hsa-miR-34c-5p	3.40E-04	-0.242122703

gene. miR-20a promotes OS metastasis by down-regulating Fas expression [12]. miR-155 involves in oncogenic regulation of OS progression such as proliferation, invasion and migration [13]. Despite these findings, the progress and development of the disease are still not clearly elucidated.

High-throughput technologies could be used for systematic researches on complex molecular processes in diseases, such as OS. Over the last two decades, many mRNA and miRNA expression studies have been performed by using microarray, a high-throughput technology to more comprehensively increase knowledge about the cellular and molecular changes in OS. However, miRNA-mRNA regulatory networks based on miRNA and mRNA expression data has not been previously elucidated. In this study, we integrated miRNA dysregulation and altered mRNA expression that occur in OS to construct identify miRNA-mRNA regulatory networks, which may provide novel insights for innovative diagnostic and treatment strategies of OS, In addition, our study would help to understand the pathology of OS.

Materials and methods

Eligible miRNA expression profiling and data preprocessing

We searched the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo) and ArrayExpress (http://www.ebi.ac.uk/arrayexpress/), for miRNA expression profiling studies in OS. We only retained miRNA expression profiling studies between OS and normal tissues by microarray. The raw microarray data was firstly downloaded from GEO and Array Express. The log2 transformation, background correction and Quantile normalization were performed for the downloaded original microarray data by MATrix LABoratory (MATLAB) software.

Differential analysis of miRNA

Based on the pretreatment results of miRNA expression values, two-tailed Student's t-test was used to identify the differently expressed miRNA in OS compared to the normal tissues. *P*-values and effect sizes of individual microarray study were obtained. *P*-values from multiple studies were combined by Fisher's method, and effect sizes from multiple studies were

hsa-miR-32-3p	3.45E-04	-0.95081882
hsa-miR-557	4.81E-04	-0.985588105
hsa-miR-146b-5p	5.77E-04	-1.562558191
hsa-miR-135a-3p	1.27E-03	-1.183892229
hsa-miR-150-5p	1.37E-03	-1.277301371
hsa-miR-26b-5p	2.15E-03	-1.943465941
hsa-miR-302d-3p	2.90E-03	-3.123108199
hsa-miR-659-3p	3.94E-03	-1.194627283
hsa-miR-335-5p	4.09E-03	-2.224509509
hsa-miR-217	4.14E-03	-2.969063299
hsa-miR-200a-5p	4.74E-03	-1.031405471
hsa-miR-148a-3p	7.52E-03	-0.15955827
hsa-miR-652-3p	9.43E-03	-2.274455133
hsa-miR-135b-3p	9.72E-03	-2.59797485

combined by the random effects model. The thresholds for differentially expressed miRNAs were *P*-value < 0.01.

Bioinformatics prediction of miRNA targets

As miRNAs function by down-regulating the expression of target genes, bioinformatics prediction of miRNA targets is important for the research of miRNA function. The target genes of differentially expressed miRNAs were predicted by 6 bioinformatic algorithms (DIANAmT, miRanda, miRDB, miRWalk, PICTAR and Targetscan) by the online tools of miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) [14].

Combining predicted targets with gene expression profiling

Due to the reversely correlated expression between miRNA and its target genes, we combined predicted targets with gene expression profiling which was available in an recently published integrated analysis of 8 microarray datasets (PMID: 25023069) [15]. The target genes recorded by ≥ 4 algorithms were selected to compare with the gene expression profiling data, and we selected microRNA-target gene pairs with opposing expression patterns to subject to further investigation [16-18].

Constructing regulatory network between miRNAs and their targets

The posttranscriptional regulatory network is defined as a directed and bipartite graph in which expressions of miRNA-target gene interacting pairs are reversely correlated. We conducted a regulatory network of miRNAs and genes in OS with the identified miRNA-target gene interacting pairs, and visualized with Cytoscape [19].

Functional annotation

To gain insights into the biological functions of miRNA target genes, we performed Gene ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO provides a common descriptive framework and functional annotation and classification for analyze the gene sets data. KEGG pathway database is a recognized and comprehensive database including all kinds of biochemistry pathways [20]. The online based software GENECODIS was utilized in this analysis [21].

Results

Differentially expressed miRNAs in OS

In this work, we collected one expression profiling study respectively in GEO database (Accession: GSE28425) and ArrayExpress (Accession: E-MTAB-1136), including 16 samples of OS and 56 samples of normal control. After normalization of the original miRNA expression profiling, we performed miRNA differential expressed analysis between OS and normal control samples using MATLAB. Finally, 59 miRNAs were regarded as significantly differentially expressed under the threshold of *P*-value < 0.01, with 28 up-regulated and 31 down-regulated miRNAs (**Table 1**).

Identification of differently expressed miRNA target genes

To know target genes of differentially expressed miRNA in OS, bioinformatics prediction was performed by miRWalk database. In addition we compared the predicted target genes recorded by ≥ 4 algorithms, to gene expression profiling data from an integrated analysis conducted by Zuozhang Yang. As a result, we identified 158 miRNA-target gene pairs for 10 up-regulated miRNA, and 15 miRNA-target gene pairs for 7 down-regulated miRNA (**Table 2**).

Regulatory network of miRNAs and target genes

The miRNA-target genes regulatory network in OS was constructed with the miRNA-target

Table 2. The 109 miRNA-target gene pairs reversely correlated with the expressions of 17 differentailly expression miRNAs

miRNA	Regulation (miRNA)	Count of targets	Target Genes
hsa-miR-124-3p	up	22	AMOTL1, BCL11A, CCND2, DDX26B, EYA4, GLI3, GNAI2, HECTD2, ITGA3, LMO4, MITF, MST4, OSBPL10, PGM1, RAB34, RARG, ROR2, SEMA6D, SERTAD4,TEAD1, VAMP3, ZFP36L1
hsa-miR-137	up	12	ABHD6, ALDH1A2, ATP1B1, DEXI, MITF, NRXN3, PLXNA2, PPARGC1A, PTGFRN, SYT1, TRPS1, ZBTB4
hsa-miR-139-5p	down	1	GALNT7
hsa-miR-148a-3p	down	3	CADM1, ELAVL2, ZNF217
hsa-miR-148b-3p	up	6	BTBD3, CFL2, KIAA1217, NPTN, PRICKLE2, RAB34
hsa-miR-149-5p	up	1	EXT1
hsa-miR-153-3p	up	8	ACTN4, AUTS2, DDIT4, EXT1, FGFR2, NPTN, PPARGC1A, ZCCHC14
hsa-miR-16-5p	down	1	SPTBN2
hsa-miR-17-5p	up	21	ACSL4, C14orf28, CCND2, CFL2,EZH1, FRMD6, HABP4, JAZF1, LAMA3, NRP2, PRRX1, PTGFRN, RAB12, RAPGEFL1, SH3PXD2A, SMOC1, SORL1, TBL1X, TRIP10, TRPS1, ZBTB4
hsa-miR-182-5p	up	9	BCL11A, BDNF, ISL1, JAZF1, KIAA1217, MITF, VAMP3, ZCCHC14, ZFP36L1
hsa-miR-195-5p	down	1	SPTBN2
hsa-miR-19b-3p	up	37	ABR, ACSL4, ACTN1, BLCAP, CALM1, CBX7, CCND2, CLIP4, DOCK3, DTNA, ETV5, FAT3, FOXP2, JAZF1, KIAA1217, LRCH2, MID1, MPPED2, MST4, NHS, NPTN, NRP2, PCDH10, PRICKLE2, RAB34, RAPGEFL1, RBMS3, SMOC1, SPRYD3, SRGAP3, ST3GAL5, SYT1, TRPS1, TSHZ3, WDR1, ZBTB4, ZNF516
hsa-miR-20a-5p	up	26	ACSL4, ANO6, C14orf28, CCND2, CFL2, CSRNP3, EZH1, FRMD6, HABP4, HECTD2, JAZF1, LAMA3, MFN2, NRP2, PLSCR4, PRRX1, PTGFRN, RAB12, RAPGEFL1, SH3PXD2A, SMOC1, SORL1, TBL1X, TRIP10, TRPS1, ZBTB4
hsa-miR-26b-5p	down	6	DAPK1, ELAVL2, SLC7A11, TBC1D4, YPEL1, ZNF217
hsa-miR-29b-3p	up	16	AMOT, ATP1B1, ATP2B4, BCL11A, CCND2, COL4A5, DGKH, GRIP1, ISL1, KIRREL, NAV2, RAB12, ROBO1, TPM1, TRIB2, ZFP36L1
hsa-miR-34c-5p	down	2	E2F5, GALNT7
hsa-miR-486-5p	down	1	ELAVL2

Table 4. KEGG pathway enrichment analysis of differential expression miRNA target genes (Top 15)

KEGG ID	KEGG term	Count	FDR	Genes
hsa04510	Focal adhesion	9	1.34E-04	TLN1, LAMA3, ACTN4, CCND2, BCL2, ACTN1, ITGA3, ITGB3, COL4A6
hsa04360	Axon guidance	6	3.14E-03	GNAI2, SEMA6D, ROBO1, PLXNA2, CFL2, SRGAP3
hsa05200	Pathways in cancer	8	1.28E-02	FGFR2, LAMA3, BCL2, MITF, RUNX1T1, ITGA3, GLI3, COL4A6
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	2.13E-02	ACTN4, ACTN1, ITGA3, ITGB3
hsa04810	Regulation of actin cytoskeleton	6	2.54E-02	FGFR2, ACTN4, CFL2, ACTN1, ITGA3, ITGB3
hsa05222	Small cell lung cancer	4	2.77E-02	LAMA3, BCL2, ITGA3, COL4A6
hsa04512	ECM-receptor interaction	4	2.77E-02	LAMA3, ITGA3, ITGB3, COL4A6
hsa04530	Tight junction	4	8.73E-02	GNAI2, ACTN4, ACTN1, AMOTL1

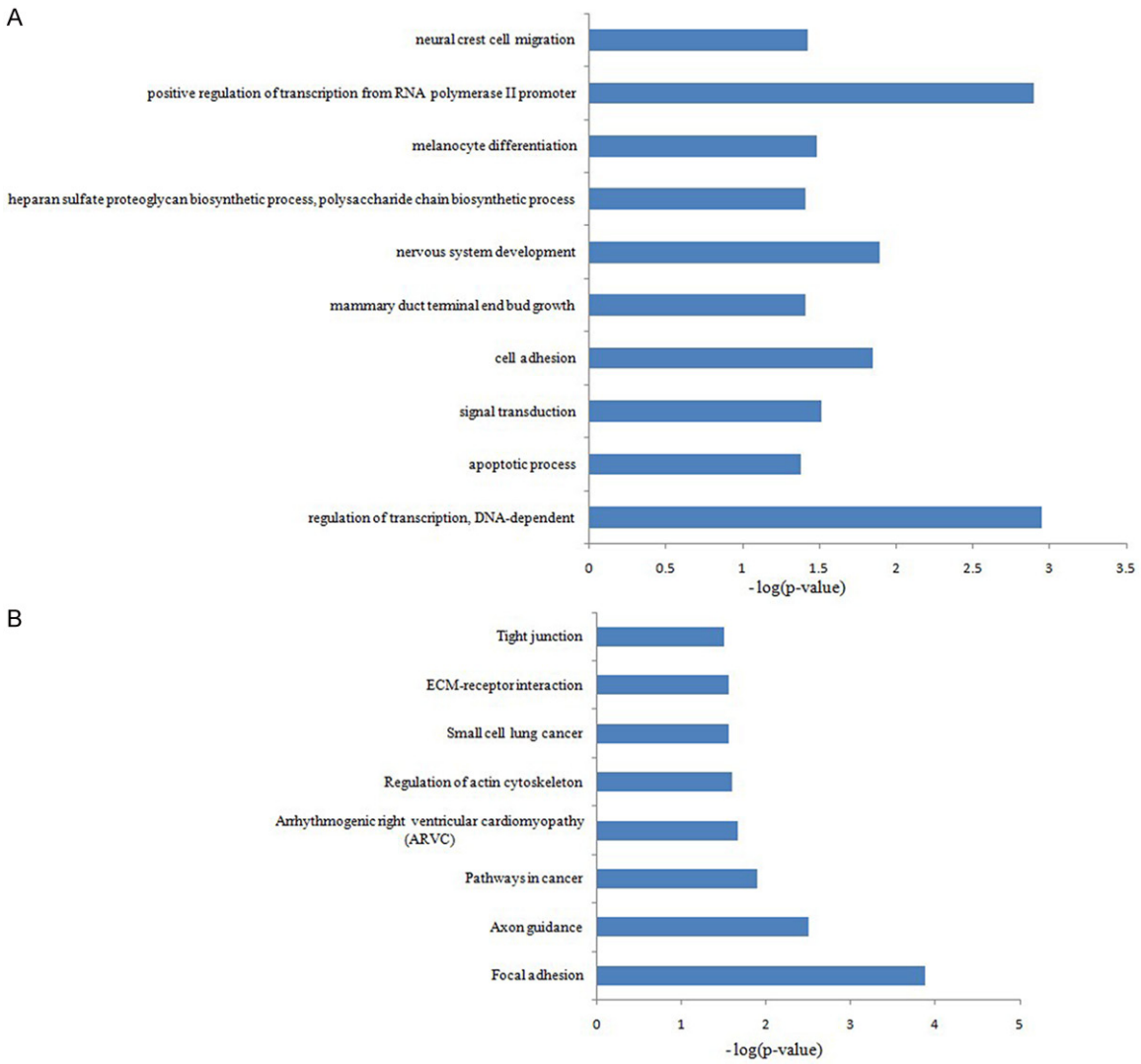

Figure 1. Significantly enriched functional annotation of differently expressed miRNA target genes. A. The top 10 enriched GO categories for biological process; B. The significantly enriched KEGG pathway.

gene pairs by Cytoscape software. As a result, 17 miRNAs and 109 differentially expressed genes formed 173 miRNA-target gene pairs with an inverse correlation of expression (**Figure 1**). Among all the differentially expressed miRNAs, miR-19b-3p had the most regulatory target genes (37 target genes) and miR-20a-5p and miR-124-3p targeted 26 and 22 differentially expressed genes. Additionally among the differentially expressed genes, CCND2 had most regulatory miRNAs (5 potential controlling miRNAs) and ZBTB4, TRPS1 and JAZF1 were regulated by 4 miRNAs. Those miRNAs targeting multiple genes and those genes targeted by multiple miRNAs, which demonstrated the nodal points of regulatory network, may play more significant roles in OS.

GO classification and KEGG pathways of miRNA target genes

GO functional and KEGG pathway enrichment analyses were performed for the 109 target gens. We found that regulation of transcription, DNA-dependent (GO: 0006355, P = 1.14E-03) and apoptotic process (GO: 0006915, P = 4.16E-02) were significantly enriched for biological processes. While for molecular functions, transcription factor activity (GO: 0003700, P = 4.46E-05) and transcription regulator activity (GO: 0030528, P = 4.63E-05) were significantly enriched, and for cellular component, cell junction (GO: 0030054, P = 1.85E-03) and cell projection (GO: 0042995, P = 2.28E-03) were significantly enriched (**Table 3**, **Figure 2A**).

Table 3. GO functional annotation of differentially expression miRNA target genes (Top 15)

GO ID	GO Term	Count	%	FDR
Biological process				
GO: 0006355	regulation of transcription, DNA-dependent	17	0.1574074	1.14E-03
GO: 0006915	apoptotic process	6	0.0555556	4.16E-02
GO: 0007165	signal transduction	10	0.0925926	3.05E-02
GO: 0007155	cell adhesion	8	0.0740741	1.43E-02
GO: 0060763	mammary duct terminal end bud growth	1	0.0092593	3.90E-02
GO: 0007399	nervous system development	7	0.0648148	1.29E-02
GO: 0015014	heparan sulfate proteoglycan biosynthetic process, polysaccharide chain biosynthetic process	1	0.0092593	3.90E-02
GO: 0030318	melanocyte differentiation	2	0.0185185	3.28E-02
GO: 0045944	positive regulation of transcription from RNA polymerase II promoter	10	0.0925926	1.28E-03
GO: 0001755	neural crest cell migration	2	0.0185185	3.79E-02
GO: 0007389	pattern specification process	3	0.0277778	3.05E-02
GO: 0000122	negative regulation of transcription from RNA polymerase II promoter	9	0.0833333	1.05E-03
GO: 0043065	positive regulation of apoptotic process	3	0.0277778	4.63E-02
GO: 0030324	lung development	3	0.0277778	2.75E-02
GO: 0007275	multicellular organismal development	14	0.1296296	3.34E-04
Molecular function				
GO: 0003700	transcription factor activity	23	16.083916	4.46E-05
GO: 0030528	transcription regulator activity	30	20.979021	4.63E-05
GO: 0008134	transcription factor binding	13	9.0909091	2.09E-03
GO: 0003682	chromatin binding	7	4.8951049	2.25E-03
GO: 0043565	sequence-specific DNA binding	14	9.7902098	2.96E-03
GO: 0042802	identical protein binding	14	9.7902098	4.64E-03
GO: 0046982	protein heterodimerization activity	7	4.8951049	1.09E-02
GO: 0016564	transcription repressor activity	8	5.5944056	2.32E-02
GO: 0003779	actin binding	8	5.5944056	2.69E-02
GO: 0005509	calcium ion binding	15	10.48951	3.44E-02
GO: 0003677	DNA binding	30	20.979021	3.50E-02
Cellular component				
GO: 0030054	cell junction	12	8.3916084	1.85E-03
GO: 0042995	cell projection	14	9.7902098	2.28E-03
GO: 0031252	cell leading edge	6	4.1958042	3.97E-03
GO: 0001725	stress fiber	3	2.0979021	1.41E-02
GO: 0005856	cytoskeleton	19	13.286713	1.51E-02
GO: 0005886	plasma membrane	40	27.972028	1.57E-02
GO: 0032432	actin filament bundle	3	2.0979021	1.65E-02
GO: 0042641	actomyosin	3	2.0979021	1.77E-02
GO: 0016323	basolateral plasma membrane	6	4.1958042	1.91E-02
GO: 0005604	basement membrane	4	2.7972028	2.14E-02
GO: 0044459	plasma membrane part	26	18.181818	2.17E-02
GO: 0005667	transcription factor complex	6	4.1958042	2.18E-02
GO: 0044451	nucleoplasm part	10	6.993007	2.49E-02
GO: 0017053	transcriptional repressor complex	3	2.0979021	3.86E-02
GO: 0005925	focal adhesion	4	2.7972028	4.24E-02

We also performed the KEGG pathway enrichment analysis for differently expressed miRNA target genes. Hypergeometric test with P value < 0.05 were used as the criteria for pathway detection. The most significant pathway in our analysis was focal adhesion (P = 1.34E-04). Furthermore, axon guidance (P = 3.14E-03) and pathways in cancer (P = 1.28E-02) are also highly enriched (**Table 4**, **Figure 2B**).

Discussion

By mediating the expression of target genes, miRNA play a critical role in the regulation of cellular biology of development and cancer. Along with bioinformatics prediction, we integrated miRNA/mRNA expression data available to generate miRNA-mRNA regulatory networks. In the present study, 59 miRNAs were found to

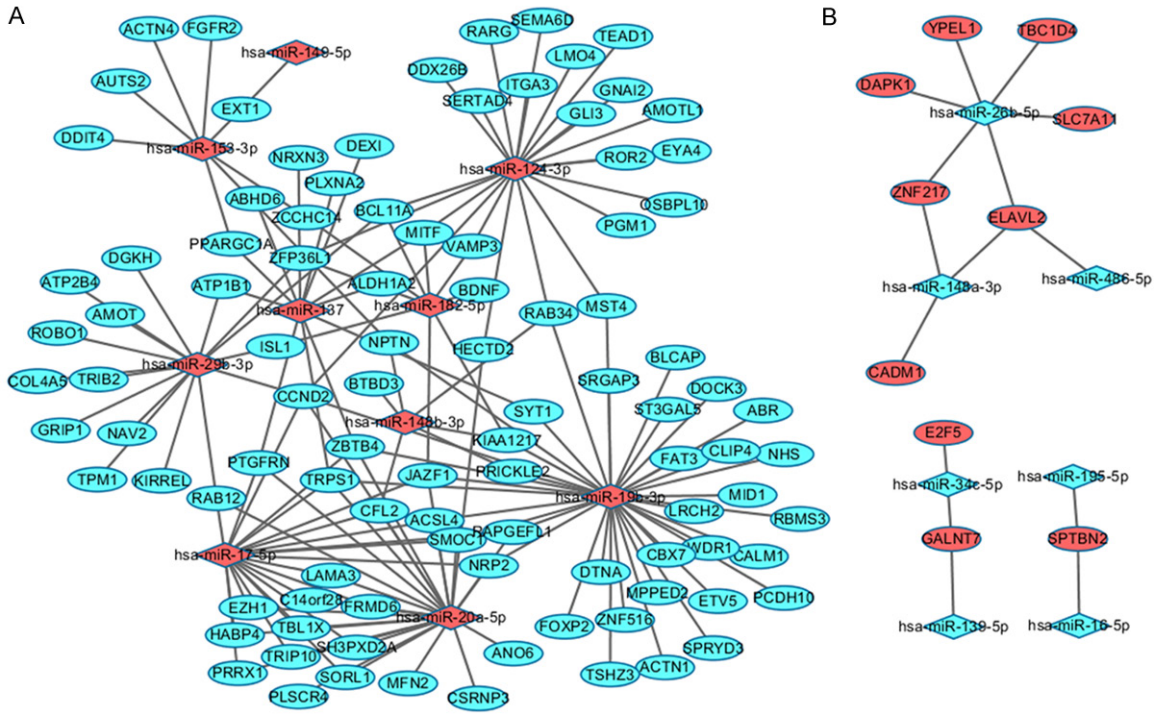

Figure 2. Regulatory network between miRNAs and target genes in osteosarcoma. The diamonds and ellipses represent the miRNAs and genes, respectively. The red and green colors represent the relatively high and low expression, respectively. The larger geometric drawing indicates the more miRNAs or genes interacted with it.

be significantly differentially expressed in the OS by integrating the acquired 2 data sets. The up-regulated miRNA with the lowest *P*-value was miR-9-3p, which has been found to regulate osteoblastic differentiation of mouse induced pluripotent stem (iPS) cells [22]. The expression of miR-9-3p altered in multiple cancers such as neuroblastoma, [23] colorectal cancer [24] and breast cancer [25], suggesting miR-9-3p was of potential importance in tumor formation and development. The down-regulated gene with the lowest *P*-value was miR-223-3p, which was significantly associated with a higher risk for progression of non-small cell lung carcinoma [26].

In addition, there are evidences showing that some genes implicated in the development of OS. Sun XH et al. discovered the altered expression of miR-646 in OS cell lines and OS tissues compared with normal osteoblast cell line. In vitro experiments showed that miR-646 regulated OS cells proliferation, migration, and invasion by targeting FGF2 [27]. miR-100-3p and miR-135b-5p were expressed differentially in OS cell lines and may be associated with the metastatic capacity of the disease [28]. Jones KB et al. found that miR-142-5p exhibit reduced expression in human OS tissues [10]. miR-486-5p was found to be down-regulated in OS cell lines relative to normal bone [29]. Shen L et al. identified a tumor-suppressive role of miR-217 in OS tumorigenesis through targeting WASF3 [30].

MiRNAs fulfil their regulatory function via targeting to corresponding genes, thus it is necessary to learn about target genes of miRNA to understand the biological functions of miRNAs. In this study we combined mRNA and miRNA expression data with bioinformatics predictions of miRNA targets via the miRWalk database to construct novel regulatory network between miRNAs and mRNAs. Consequently, 17 miRNAs and 109 genes formed 173 miRNA-target gene pairs with an inverse correlation of expression. miR-19b-3p was connected with the most regulatory target genes. miR-20a-5p and miR-124-3p regulated 26 and 22 target genes. Leung CM demonstrated that miR-19b-3p and miR-20a-5p, members of miR-17-92 cluster which has been determined to play an oncogenic role in tumorigenesis, exhibited differential responses to single-dose (SD) or mul-

tifractionated radiation in human breast cancer cells [31]. miR-124-3p exhibited altered expression in several kinds of cancers including glioma, oral squamous cell carcinomas, hepatocellular carcinoma and breast cancer [32-35], suggesting that its function is related to carcinogenesis.

Additionally in the miRNAs and mRNA regulatory network, CCND2 had most regulatory miRNAs including miR-124-3p, miR-17-5p, miR-19b-3p, miR-20a-5p, miR-29b-3p and the top 3 miRNA with the most regulatory target genes were contained. CCND2, located at chromosome 12p13, plays a key role in cell cycle G1/S transition by regulating phosphorylation of the tumor suppressor protein Rb [36]. DNA copy number alterations of CCND2 showed remarkable enhancement in OS metastatic lesion compared to a primary lesion by array comparative genomic hybridization analysis, leading to overexpression of CCND2 in OS [37, 38]. CCND2 may be considered a therapy target for OS.

Finally, through GO and pathway analysis of putative targets of miRNA we found that some of the biological function may be cancer-related including regulation of transcription, DNA-dependent, apoptotic process, signal transduction and cell adhesion. The most significant pathway in our analysis was focal adhesion, which plays a fundamental role in carcinogenesis, tumor progression and metastasis. Amounts of focal adhesion molecules including integrins, integrin-associated proteins and growth factor were found to be deregulated in several kinds of cancer [39]. Many of the differently expressed target genes identified in this study were involved in pathways in cancer, including FGFR2, LAMA3, BCL2, MITF, RUNX1T1, ITGA3, GLI3 and COL4A6 as cancer suppressors or oncogenes.

In this study, differentially expressed miRNA were identified between OS and normal tissues by combining OS miRNA expression data sets available. Based on a published integrated study, 109 miRNA target genes found to be anticorrelated with miRNA expressions in OS. A novel miRNA-target gene regulatory network was constructed with the miRNA-target gene pairs. miR-19b-3p, miR-20a-5p, miR-124-3p and their common target CCND2, the nodal points of regulatory network, may play important roles in OS. Bioinformatics analysis of biological functions and pathways demonstrated that target genes of miRNAs are highly correlated with carcinogenesis. Our findings may help to understand the molecular mechanisms of OS and identify targets of effective targeted therapies for OS. Further functional experiments may provide additional insights into the role of the differentially regulated miRNAs in the development of OS.

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Disclosure of conflict of interest

None.

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References

[1] PosthumaDeBoer J, Witlox MA, Kaspers GJ and van Royen BJ. Molecular alterations as target for therapy in metastatic osteosarcoma: a review of literature. Clin Exp Metastasis 2011; 28: 493-503.

[2] O'Day K and Gorlick R. Novel therapeutic agents for osteosarcoma. Expert Rev Anticancer Ther 2009; 9: 511-523.

[3] Winter J and Diederichs S. MicroRNA biogenesis and cancer. Methods Mol Biol 2011; 676: 3-22.

[4] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC and Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006; 103: 2257-2261.

[5] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.

[6] Doench JG and Sharp PA. Specificity of microRNA target selection in translational repression. Genes Dev 2004; 18: 504-511.
[7] Boehm A, Neff J, Squire J, Bayani J, Nelson M and Bridge J. Cytogenetic findings in 36 osteosarcoma specimens and a review of the literature. Fetal Pediatr Pathol 2000; 19: 359-376.
[8] Maire G, Martin JW, Yoshimoto M, Chilton-MacNeill S, Zielenska M and Squire JA. Analysis of miRNA-gene expression-genomic profiles reveals complex mechanisms of microRNA deregulation in osteosarcoma. Cancer Genet 2011; 204: 138-146.
[9] Baumhoer D, Zillmer S, Unger K, Rosemann M, Atkinson MJ, Irmler M, Beckers J, Siggelkow H, von Luettichau I, Jundt G, Smida J and Nathrath M. MicroRNA profiling with correlation to gene expression revealed the oncogenic miR-17-92 cluster to be up-regulated in osteosarcoma. Cancer Genet 2012; 205: 212-219.
[10] Jones KB, Salah Z, Del Mare S, Galasso M, Gaudio E, Nuovo GJ, Lovat F, LeBlanc K, Palatini J, Randall RL, Volinia S, Stein GS, Croce CM, Lian JB and Aqeilan RI. miRNA signatures associate with pathogenesis and progression of osteosarcoma. Cancer Res 2012; 72: 1865-1877.
[11] Lulla RR, Costa FF, Bischof JM, Chou PM, de FBM, Vanin EF and Soares MB. Identification of Differentially Expressed MicroRNAs in Osteosarcoma. Sarcoma 2011; 2011: 732690.
[12] Huang G, Nishimoto K, Zhou Z, Hughes D and Kleinerman ES. miR-20a encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas expression. Cancer Res 2012; 72: 908-916.
[13] Lv H, Guo J, Li S and Jiang D. inhibitor reduces the proliferation and migration in osteosarcoma MG-63 cells. Exp Ther Med 2014; 8: 1575-1580.
[14] Dweep H, Sticht C, Pandey P and Gretz N. miRWalk--database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J Biomed Inform 2011; 44: 839-847.
[15] Yang Z, Chen Y, Fu Y, Yang Y, Zhang Y and Li D. Meta-analysis of differentially expressed genes in osteosarcoma based on gene expression data. BMC Med Genet 2014; 15: 80.
[16] Luo Z, Zhang L, Li Z, Li X, Li G, Yu H, Jiang C, Dai Y, Guo X, Xiang J and Li G. An in silico analysis of dynamic changes in microRNA expression profiles in stepwise development of nasopharyngeal carcinoma. BMC Med Genomics 2012; 5: 3.
[17] Lionetti M, Biasiolo M, Agnelli L, Todoerti K, Mosca L, Fabris S, Sales G, Deliliers GL, Bicciato S, Lombardi L, Bortoluzzi S and Neri A. Identification of microRNA expression patterns and definition of a microRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma. Blood 2009; 114: e20-26.
[18] Enerly E, Steinfeld I, Kleivi K, Leivonen SK, Aure MR, Russnes HG, Ronneberg JA, Johnsen H, Navon R, Rodland E, Makela R, Naume B, Perala M, Kallioniemi O, Kristensen VN, Yakhini Z and Borresen-Dale AL. miRNA-mRNA integrated analysis reveals roles for miRNAs in primary breast tumors. PLoS One 2011; 6: e16915.
[19] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
[20] Altermann E and Klaenhammer TR. PathwayVoyager: pathway mapping using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. BMC Genomics 2005; 6: 60.
[21] Tabas-Madrid D, Nogales-Cadenas R and Pascual-Montano A. GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. Nucleic Acids Res 2012; 40: W478-483.
[22] Okamoto H, Matsumi Y, Hoshikawa Y, Takubo K, Ryoke K and Shiota G. Involvement of microRNAs in regulation of osteoblastic differentiation in mouse induced pluripotent stem cells. PLoS One 2012; 7: e43800.
[23] Murray MJ, Raby KL, Saini HK, Bailey S, Wool SV, Tunnacliffe JM, Enright AJ, Nicholson JC and Coleman N. Solid tumors of childhood display specific serum microRNA profiles. Cancer Epidemiol Biomarkers Prev 2015;24: 350-60.
[24] Slattery ML, Herrick JS, Mullany LE, Valeri N, Stevens J, Caan BJ, Samowitz W and Wolff RK. An evaluation and replication of miRNAs with disease stage and colorectal cancer-specific mortality. Int J Cancer 2014; [Epub ahead of print].
[25] Zawistowski JS, Nakamura K, Parker JS, Granger DA, Golitz BT and Johnson GL. MicroRNA 9-3p targets beta1 integrin to sensitize claudin-low breast cancer cells to MEK inhibition. Mol Cell Biol 2013; 33: 2260-2274.
[26] Sanfiorenzo C, Ilie MI, Belaid A, Barlesi F, Mouroux J, Marquette CH, Brest P and Hofman P. Two panels of plasma microRNAs as non-invasive biomarkers for prediction of recurrence in resectable NSCLC. PLoS One 2013; 8: e54596.
[27] Sun XH, Geng XL, Zhang J and Zhang C. miRNA-646 suppresses osteosarcoma cell metastasis by downregulating fibroblast growth factor 2 (FGF2). Tumour Biol 2015; 36: 2127-34.
[28] Lauvrak SU, Munthe E, Kresse SH, Stratford EW, Namlos HM, Meza-Zepeda LA and Myklebost O. Functional characterisation of osteosarcoma cell lines and identification of mRNAs

and miRNAs associated with aggressive cancer phenotypes. Br J Cancer 2013; 109: 2228-2236.
[29] Namlos HM, Meza-Zepeda LA, Baroy T, Ostensen IH, Kresse SH, Kuijjer ML, Serra M, Burger H, Cleton-Jansen AM and Myklebost O. Modulation of the osteosarcoma expression phenotype by microRNAs. PLoS One 2012; 7: e48086.
[30] Shen L, Wang P, Yang J and Li X. MicroRNA-217 regulates WASF3 expression and suppresses tumor growth and metastasis in osteosarcoma. PLoS One 2014; 9: e109138.
[31] Leung CM, Chen TW, Li SC, Ho MR, Hu LY, Liu WS, Wu TT, Hsu PC, Chang HT and Tsai KW. MicroRNA expression profiles in human breast cancer cells after multifraction and single-dose radiation treatment. Oncol Rep 2014; 31: 2147-2156.
[32] Xia H, Cheung WK, Ng SS, Jiang X, Jiang S, Sze J, Leung GK, Lu G, Chan DT, Bian XW, Kung HF, Poon WS and Lin MC. Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. J Biol Chem 2012; 287: 9962-9971.
[33] Hunt S, Jones AV, Hinsley EE, Whawell SA and Lambert DW. MicroRNA-124 suppresses oral squamous cell carcinoma motility by targeting ITGB1. FEBS Lett 2011; 585: 187-192.
[34] Furuta M, Kozaki KI, Tanaka S, Arii S, Imoto I and Inazawa J. miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. Carcinogenesis 2010; 31: 766-776.
[35] Liang YJ, Wang QY, Zhou CX, Yin QQ, He M, Yu XT, Cao DX, Chen GQ, He JR and Zhao Q. MiR-124 targets Slug to regulate epithelial-mesenchymal transition and metastasis of breast cancer. Carcinogenesis 2013; 34: 713-722.
[36] Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995; 81: 323-330.
[37] Kanamori M, Sano A, Yasuda T, Hori T and Suzuki K. Array-based comparative genomic hybridization for genomic-wide screening of DNA copy number alterations in aggressive bone tumors. J Exp Clin Cancer Res 2012; 31: 100.
[38] Di Fiore R, Fanale D, Drago-Ferrante R, Chiaradonna F, Giuliano M, De Blasio A, Amodeo V, Corsini LR, Bazan V, Tesoriere G, Vento R and Russo A. Genetic and molecular characterization of the human osteosarcoma 3AB-OS cancer stem cell line: a possible model for studying osteosarcoma origin and stemness. J Cell Physiol 2013; 228: 1189-1201.
[39] Eke I and Cordes N. Focal adhesion signaling and therapy resistance in cancer. Semin Cancer Biol 2015; 31C: 65-75.