Int J Clin Exp Med 2016;9(8):15345-15353
www.ijcem.com /ISSN:1940-5901/IJCEM0029430

Original Article

Interactions of *ABLIMI* and *CXCL5* with miRNAs as a prognostic indicator for clinical outcome of osteosarcoma

Pengfei Gao[1], Zhaowei Teng[2], Iixin Ji[1], Wengui Xie[1]

[1]*Department of Spinal Surgery, North Medical District of Linyi People's Hospital Group, Linyi, China;* [2]*Department of Orthopedic Surgery, People's Hospital of Yuxi City, The 6th Affiliated Hospital of Kunming Medical College, Yuxi, China*

Received March 29, 2016; Accepted July 10, 2016; Epub August 15, 2016; Published August 30, 2016

Abstract: Osteosarcoma (OS) is a kind of high-grade bone-forming malignancy with poor prognosis, causing huge economic losses to the patients and society. Identification of novel biomarkers could be beneficial for the diagnosis and prognosis of OS patients. We aimed to explore the molecular mechanism of osteosarcoma by the differentially expressed miRNAs and mRNAs screened out by microarray data together with the function and metabolic pathway analysis of target genes. Expression data of miRNA and mRNA were downloaded from The Genome Expression Omnibus (GEO) dataset. Differentially expressed miRNAs of osteosarcoma were screened out by two-fold principle. Target genes of differentially expressed miRNAs were extracted out by miRTarBase software. Besides, DAVID dataset was used for the functional annotation. Differentially expressed genes of mRNA microarray were screened out for the study of relation between miRNA and mRNA. A total of 33 differentially expressed miRNAs were screened out, including 27 up-regulated miRNAs and 6 down-regulated miRNAs. Three miRNAs, hsa-miR-182, hsa-miR-486-5p and hsa-miR760, were found to be the most differentially expressed ones, which were all up-regulated and could be used to differentiate osteosarcoma tissues from the normal ones. Besides, two up-regulated genes and fifteen significant down-regulated genes were identified by analysis of mRNA microarray data. Two target genes, the up-regulated gene, *ABLIM1* (actin binding LIM protein 1) and the down-regulated gene, *CXCL5* (chemokine (C-X-C motif) ligand 5) were recognized as the critical genes. We found that miRNAs interacting with *ABLIM1* were hsa-miR-373-3p and hsa-miR-302e while miRNAs interacting with *CXCL5* was hsa-miR-3655. The screened miRNAs and critical genes, together with the interactions, could help in better understanding the role of miRNA and mRNAin the occurrence and development of osteoporosis, providing new insights into the treatment of osteosarcoma.

Keywords: Osteosarcoma, miRNA, mRNA, biomarker, pathway analysis

Introduction

Osteosarcoma (OS) is a kind of most common primary malignant bone tumor. Adolescents and young adults, with the age ranging from 15 to 39 years, are more susceptible to osteosarcoma than other people [1-3]. Survival rate of OS seem to reach the plateau despite with the improvement of survival rate thanks to the chemotherapy strategies, Currently, the survival time of 65% patients is five years [4] and it left the problem of significant chemotherapy-related toxicity after treatment [5]. What's worse, there was still a large number of patients who cannot exempt from amputation and even die due to lung metastasis or relapse [6], causing a huge economic burden to families of patients and the society. Besides, some prior studies demonstrated that a significant risk for OS patients with increasing age was associated with the poorer prognosis of the disease [7, 8]. Therefore, there is still urgent need for the development of new markers that could be helpful for the prediction, therapeutic modalities and the prognostic development of OS disease [4, 9]. Therefore, in this study, we aimed to identify the differentially expressed miRNAs and mRNAs between OS patients and normal ones, with the attempt to find the new markers and therapy targets of OS.

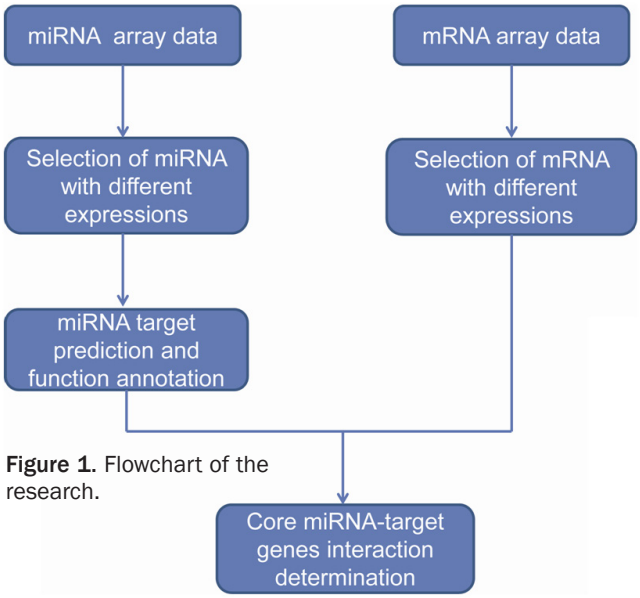

Figure 1. Flowchart of the research.

Recent studies showed that DNA damage would lead to genomic instability, malignant transformation, and cell death [6]. MicroRNAs are a series of non-coding RNA molecules that can negatively regulate gene expression and play import roles in various biological processes [10]. Increasing evidence has demonstrated that miRNAs are associated with several types of tumor by regulating cell migration and invasion. DNA microarray technology was used in this study to determine the differentially expressed miRNAs and mRNAs of OS. The new technology allows us to screen tens of thousands of genes simultaneously and be able to look at the molecular mechanism of disease development on a whole genome scale and the method has been used in many studies to identify genes. However, since there has been few public databases built to facilitate data sharing [11-13], it remains a big problem to integrate data from different organism in order to make a reliable hypotheses.

In this study, three significantly up-regulated miRNAs, has-miR-182, has-miR-486-5p and has-miR-760, was identified, which may play important roles in the development and progression of OS. What's more, two genes were found to be regulated by related miRNAs in the interaction analysis, the up-regulated gene *ABLIMI* was regulated by has-miR-373-3p and has-miR-302e while the down-regulated gene *CXCL5* was regulated by has-miR-3655, suggesting their potential role as a novel diagnostic and prognostic marker of OS.

Materials and methods

Data source of miRNA and mRNA data of osteosarcoma

The microarray expression data profiling of miRNA and mRNA were downloaded from the Genome expression omnibus (GEO) dataset with the accession number GSE70415. A total of six samples, five osteosarcoma cell lines (MG63, Saos, HOS, NY, Hu09) and one normal mesenchymal stem cells sample (hMSC), were included. The whole datasets was mainly composed of two subsets. The expression dataset of miRNA with osteosarcoma was GSE70367 with the platform of GPL16384 ([miRNA-3] Affymetrix Multispecies miRNA-3 Array) while the mRNA expression data of osteosarcoma was GSE70414 with the platform of GPL570 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array).

Differentially expressed miRNA screening

Firstly, information of mature miRNA matching a single probe out of genome from GPL 16384 was screened out. Then, the expression level of normal and osteosarcoma samples of these mature miRNAs was extracted out. Differentially expressed miRNAs were screened out by two-fold principle. To be more specific, if the expression level of miRNA in osteosarcoma was two folds or more than the normal samples, we assumed that these miRNAs were significantly up-regulated in osteosarcoma samples. On the contrary, if the expression level of miRNA in normal samples was two folds or more than the osteosarcoma specimens, we assumed that these miRNAs were significantly down-regulated in osteosarcoma samples. The difference was assumed not significant in other circumstances.

Cluster analysis and principal component analysis of differentially expressed miRNAs in ostersarcoma

The heatmap of differentially expressed miRNAs of osteosarcoma were drawn by heatmap package in R language. The most differentially expressed miRNAs between osteosarcoma samples and normal specimens were determined by the heatmap. Then, principal component analysis (PCA) was performed on these miRNAs by the expression value in different

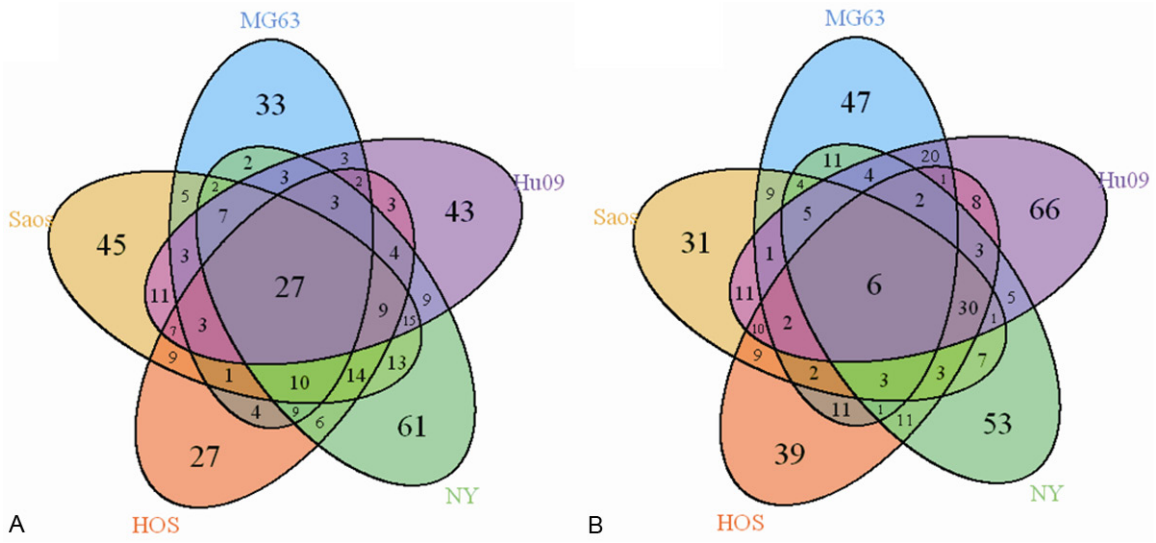

Figure 2. The up-regulated miRNA (A) and down-regulated miRNA (B) between osteosacoma samples and controls. MG63, Saos, HOS, NY and Hu09 were used as five cell lines representing osteosarcoma. Expression changes with two-folds was used as criterion for differentially expressed miRNAs screening. A total of 27 up-regulated miRNAs and 6 down-regulated miRNAs in the five osteosarcoma cell lines were found out.

Table 1. Information of up-regualted miRNA and down-regulated miRNA of osteosarcoma samples compared to control specimens

miRNA	Origin	Position	Sequence
up-regulated			
hsa-miR-1273d	hsa-mir-1273d	1:10287776-10287861 (+)	GAACCCAUGAGGUUGAGGCUGCAGU
hsa-miR-135b-star	hsa-mir-135b	1:205417430-205417526 (-)	AUGUAGGGCUAAAAGCCAUGGG
hsa-miR-139-3p	hsa-mir-139	11:72326107-72326174 (-)	GGAGACGCGGCCCUGUUGGAGU
hsa-miR-1538	hsa-mir-1538	16:69599711-69599771 (-)	CGGCCCGGGCUGCUGCUGUUCCU
hsa-miR-182	hsa-mir-182	7:129410223-129410332 (-)	UUUGGCAAUGGUAGAACUCACACU
hsa-miR-200a-star	hsa-mir-200a	1:1103243-1103332 (+)	CAUCUUACCGGACAGUGCUGGA
hsa-miR-208b	hsa-mir-208b	14:23887196-23887272 (-)	AUAAGACGAACAAAAGGUUUGU
hsa-miR-302e	hsa-mir-302e	11:7255997-7256068 (+)	UAAGUGCUUCCAUGCUU
hsa-miR-3176	hsa-mir-3176	16:593277-593366 (+)	ACUGGCCUGGGACUACCGG
hsa-miR-3655	hsa-mir-3655	5:140027429-140027511 (+)	GCUUGUCGCUGCGGUGUUGCU
hsa-miR-3682-5p	hsa-mir-3682	2:54076259-54076342 (-)	CUACUUCUACCUGUGUUAUCAU
hsa-miR-3692	hsa-mir-3692	6:157950164-157950232 (+)	GUUCCACACUGACACUGCAGAAGU
hsa-miR-373-star	hsa-mir-373	19:54291959-54292027 (+)	ACUCAAAAUGGGGGCGCUUUCC
hsa-miR-4275	hsa-mir-4275	4:28821204-28821290 (+)	CCAAUUACCACUUCUUU
hsa-miR-4295	hsa-mir-4295	10:114393929-114394013 (+)	CAGUGCAAUGUUUUCCUU
hsa-miR-4322	hsa-mir-4322	19:10341089-10341161 (+)	CUGUGGGCUCAGCGCGUGGGG
hsa-miR-4323	hsa-mir-4323	19:42637597-42637665 (-)	CAGCCCCACAGCCUCAGA
hsa-miR-4504	hsa-mir-4504	14:50766573-50766664 (-)	UGUGACAAUAGAGAUGAACAUG
hsa-miR-451b	hsa-mir-451b	17:27188389-27188456 (+)	UAGCAAGAGAACCAUUACCAUU
hsa-miR-4785	hsa-mir-4785	2:161264321-161264393 (-)	AGAGUCGGCGACGCCGCCAGC
hsa-miR-4791	hsa-mir-4791	3:19356340-19356423 (-)	UGGAUAUGAUGACUGAAA
hsa-miR-486-5p	hsa-mir-486	8:41517959-41518026 (-)	UCCUGUACUGAGCUGCCCCGAG
hsa-miR-518d-3p	hsa-mir-518d	19:54238131-54238217 (+)	CAAAGCGCUUCCCUUUGGAGC
hsa-miR-523-star	hsa-mir-523	19:54201639-54201725 (+)	CUCUAGAGGGAAGCGCUUUCUG

hsa-miR-588	hsa-mir-588	6:126805777-126805859 (+)	UUGGCCACAAUGGGUUAGAAC
hsa-miR-602	hsa-mir-602	9:140732871-140732968 (+)	GACACGGGCGACAGCUGCGGCCC
hsa-miR-760	hsa-mir-760	1:94312388-94312467 (+)	CGGCUCUGGGUCUGUGGGGA
down-regulated			
hsa-miR-199b-5p	hsa-mir-199b	9:131007000-131007109 (-)	CCCAGUGUUUAGACUAUCUGUUC
hsa-miR-323-3p	hsa-mir-323	14:101492069-101492154 (+)	CACAUUACACGGUCGACCUCU
hsa-miR-34a-star	hsa-mir-34a	1:9211727-9211836 (-)	CAAUCAGCAAGUAUACUGCCCU
hsa-miR-424	hsa-mir-424	X:133680644-133680741 (-)	CAGCAGCAAUUCAUGUUUUGAA
hsa-miR-4757-3p	hsa-mir-4757	2:19548190-19548266 (+)	CAUGACGUCACAGAGGCUUCGC
hsa-miR-875-3p	hsa-mir-875	8:100549014-100549089 (-)	CCUGGAAACACUGAGGUUGUG

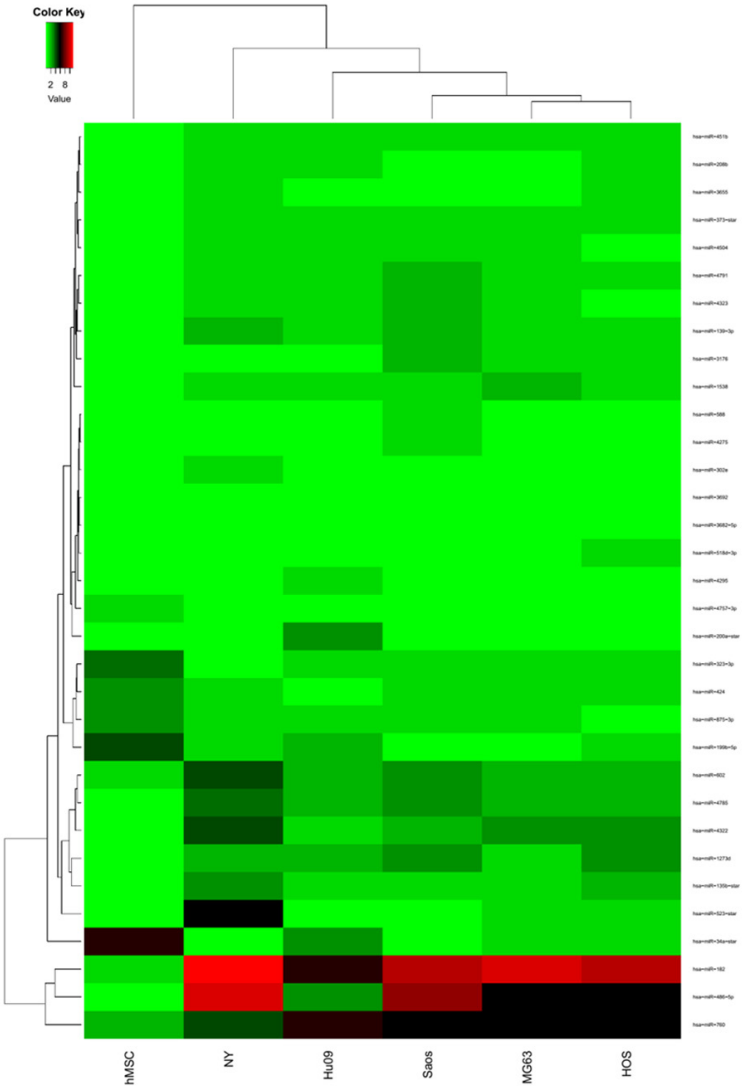

Figure 3. Cluster analysis of differentially expressed miRNAs of osteosarcoma. The horizontal axis represents the selected samples and hMSC was the control sample on the separated branch. The vertical axis stands for the result of cluster analysis of differentially expressed miRNA. Three most significant miRNAs, hsa-miR-182, hsa-miR-486-5p and hsa-miR760, were clustered into one branch, indicating their important role in the occurrence of osteosarcoma disease.

samples, thus we can determine whether these miRNAs can clearly differentiate osteosarcoma samples from normal tissues.

Prediction of target genes of differentially expressed miRNA and functional enrichment analysis

Target genes of differentially expressed miRNA verified by experiment were extracted out by miRTarBase database [14]. Then, Go (Gene Ontology) enrichment analysis [15] and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis [16] were conducted by DAVID (Database for Annotation, Visualization, and Integrated Discovery) [17]. In GO enrichment analysis, the *p* value justified after multi-testing by DAVID software was used as the threshold and *p* value less than 0.05 was extracted out.

Screening of differentially expressed mRNA

Differentially expressed mRNA in osteosarcoma samples were determined by two folds condition and these mRNAs were mapped to the above interaction of miRNA-target genes and mRNA, thus critical genes and interactions associated with osteosarcoma were screened out (**Figure 1**).

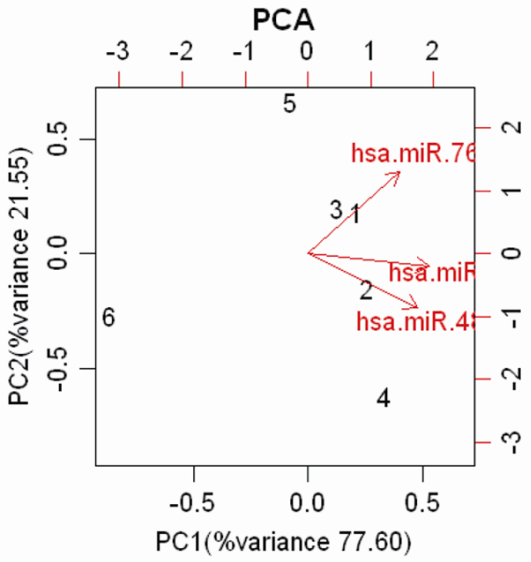

Figure 4. Principal Component Analysis (PCA) of differentially expressed miRNAs of osteosarcoma. The main role of PCA was to decrease the dimensions. PCA was performed on the three most significant miRNAs, hsa-miR-182, hsa-miR-486-5p and hsa-miR760. The accumulative contribution rate of the first and second dimension was 99.15%. In the figure, 1-5 represents osteosarcoma samples while 6 was the control one.

Results

Screening of differentially expressed miRNA in osteosarcoma

Five osteosarcoma cell lines (MG63, Saos, HOS, NY, Hu09) and one control group (hMSC) were included in our downloaded miRNA expression data with the accession number (GSE70415). The up and down-regulated miRNAs were determined between osteosarcoma samples and normal specimens. To be more specific, if the expression value of some miRNA in osteosarcoma samples was at least two folds than normal specimens, we assumed that it was up-regulated in osteosarcoma samples; if the expression value of some miRNA in normal samples was at least two folds than osteosarcoma specimens, we assumed that it was down-regulated in osteosarcoma samples. The usual and specific up and down-regulated miRNAs in osteosarcoma cells were shown in **Figure 2**.

A total of 1591 miRNAs had the single probe after excluding miRNAs originated from more than one miRNA and these miRNAs were used for the screening of differentially expressed miRNAs. The up and down-regulated miRNAs were display by the form of Wine figure (**Figure 2**) and list table (**Table 1**). As can be seen from **Figure 2**, a total of 393miRNAs were up-regulated in osteosarcoma tissue and 27 among them were up-regulated in all osteosarcoma samples. As for the down-regulated condition, a total of 417 miRNAs were down-regulated in osteosarcoma samples and six among them were down-regulated in all osteosarcoma samples.

Cluster analysis and principal component analysis of differentially expressed miRNA in osteosarcoma

Heatmap was drawn based on the expression value of the 33 differentially expressed miRNAs and we found that the control group (hMSC) was on one single branch while the osteosarcoma was on the other. Meanwhile, the result of cluster analysis of differentially expressed miRNAs showed that three miRNAs that was most differentially expressed were on the same branch and thesemiRNAs were hsa-miR-182, hsa-miR-486-5p and hsa-miR760 (**Figure 3**). Therefore, we assumed that the three differentially expressed miRNAs were most obvious in the occurrence of osteosarcoma disease.

We conducted principal component analysis of miRNA expression value on the top three significantly differentially expressed miRNAs and we found that one dimension principal component had contribution rate of 77.6% while the two dimensions had the contribution rate of 21.55%. The accumulative contributions ratio of the two was 99.15% (**Figure 4**), which indicated that these three miRNAs can differentiate osteosarcoma samples perfectly from the control group perfectly.

Prediction of target genes of differentially expressed miRNAs in osteosarcoma and functional annotation

Firstly, we extracted the target genes of differentially expressed miRNAs testified by Mirtarbase and 2048 target genes corresponding with 28 miRNAs were obtained. The number of target genes corresponding with up-regulated miRNAs was 1866 and the number of target genes matching with down-regulated miRNAs was 67. Functional enrichment annotation was

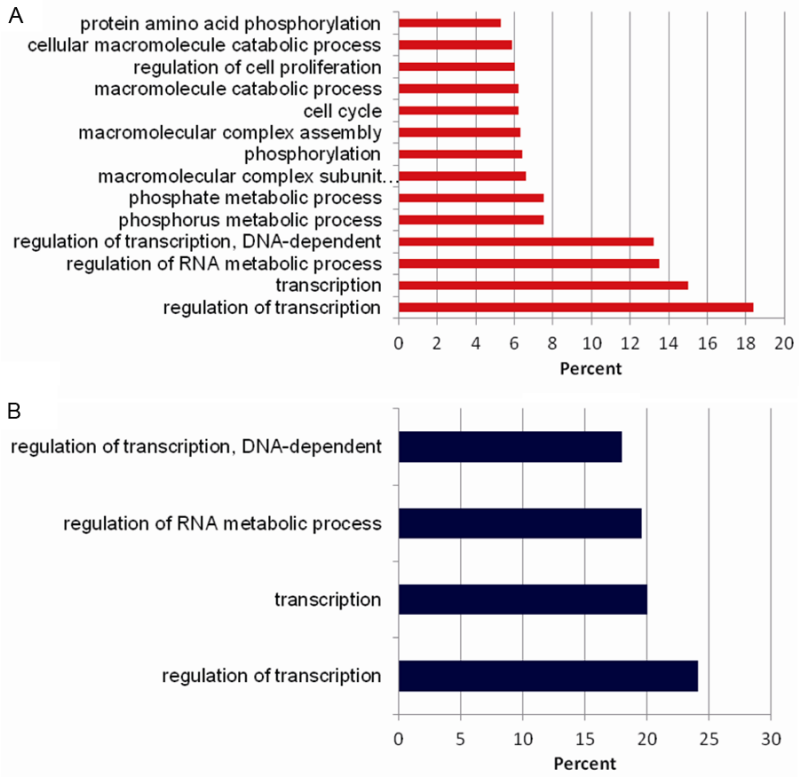

Figure 5. GO enrichment analysis of target genes of differentially expressed miRNA. The horizontal axis represents the rate of genes while the vertical axis stands for the GO terms. The red color represents the result of GO enrichment analysis result of target genes of up-regulated miRNA while the deep blue color represents the GO enrichment analysis of the target genes of down-regulated miRNAs. Both were mostly enriched in the transcriptional regulation process.

performed on these target genes and the result of GO enrichment analysis showed that the enriched GO terms of up-regulated miRNAs was much more than that of down-regulated miRNAs. Meanwhile, both target genes of up and down-regulated miRNAs were significantly enriched in the process of transcriptional regulation, confirming the transcriptional regulation role of miRNA in the occurrence of osteosarcoma disease. KEGG enrichment analysis was further conducted on these target genes of up and down-regulated miRNAs. We found that these target genes were significantly enriched in four metabolic pathways respectively and these pathways were all in high correlation with the occurrence of cancer (**Figure 5** and **Table 2**).

Screening of differentially genes between osteosarcoma samples and normal samples

Two folds principle was as well used in the determination of differentially expressed mRNA between osteosarcoma samples and normal specimens. By the comparison with the normal samples, two significant up-regulated mRNA in all five osteosarcoma cells were selected while 15 significant down-regulated mRNA were found (**Figure 6**).

The result of differentially expressed mRNA was mapped to the interaction network of miRNA-target genes mRNA. Two gene, that is, *ABLIM1* (actin binding LIM protein 1), up-regulated in osteosarcoma tissue and *CXCL5* (chemokine (C-X-C motif) ligand 5), down-regulated in osteosarcoma, were regulated by relative miRNAs. hsa-miR-373-3p and hsa-miR-302e interacted with *ABLIM1* gene while hsa-miR-3655 was in the interaction with *CXCL5* gene.

Discussion

In this study, five osteosarcoma cell lines, MG63, Saos, HOS, NY, Hu09, were used to screen the differentially expressed miRNAs. Genetic, phenotypic and functional characteristics have shown that cell lines can represent osteosarcoma clinical samples robustly [18-20]. As a result, a total of 27 miRNAs were found to be up-regulated in all five cell line while the number of down-regulated miRNAs was six. Three miRNAs, that is, hsa-miR-182, hsa-miR-486-5p and hsa-miR760 were significantly up-regulated in osteosarcoma samples, indicating their potential significant role in the progression of the disease. It's been reported that dysregulation of miR-183 significantly impacts tumor metastasis in osteosarcoma via Ezrin [21, 22], however, as for the role of miR-182 in tumoregenesis and metastasis, there hasn't been much report. The discovery of miR-182 in this study indicated a possible new marker of OS, which needs further research for the specific mechanism. Besides, the result of GO enrichment analysis revealed that target genes

Table 2. KEGG pathway enrichment analysis of target genes of up-regulated miRNAs

Category	Term	Count	%	*P*-Value	Fold Enrichment	Benjamini
up-regulated						
KEGG_PATHWAY	Systemic lupus erythematosus	28	1.5	2.00E-06	2.7	3.80E-04
KEGG_PATHWAY	Colorectal cancer	21	1.1	3.30E-04	2.4	3.10E-02
KEGG_PATHWAY	Endocytosis	35	1.9	6.80E-04	1.8	4.10E-02
KEGG_PATHWAY	Adherens junction	19	1	8.30E-04	2.3	3.80E-02
down-regulated						
KEGG_PATHWAY	Endometrial cancer	6	2.4	5.90E-04	8.5	6.20E-02
KEGG_PATHWAY	Prostate cancer	7	2.9	1.10E-03	5.8	5.80E-02
KEGG_PATHWAY	Pathways in cancer	13	5.3	1.20E-03	2.9	4.10E-02
KEGG_PATHWAY	Focal adhesion	10	4.1	1.30E-03	3.7	3.40E-02

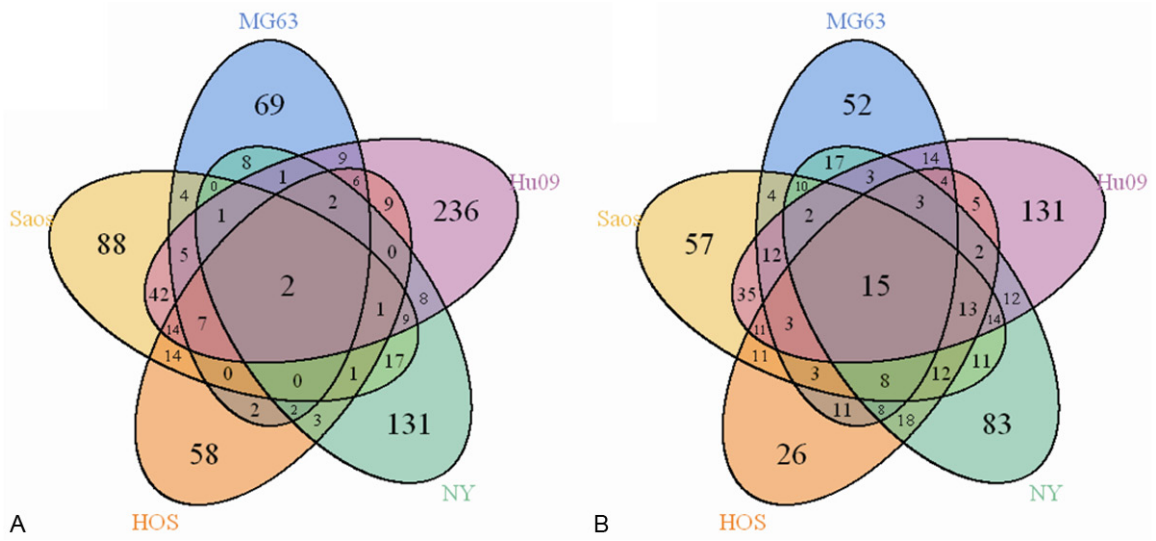

Figure 6. The up-regulated mRNA and down-regulated mRNA in OS samples. MG63, Saos, HOS, NY and Hu09, were used as five cell lines representing osteosarcoma. Expression changes with two-folds was used as criterion for differentially expressed mRNAs screening. The number of up-regulated mRNA was two while the number of down-regulated mRNA was fifteen edmiRNAs.

of differentially expressed miRNAs were mainly enriched in four pathways, that is, regulation of transcription, DNA dependent, regulation of RNA metabolic process, transcription and regulation of transcription, all in high relevance with the occurrence of cancer, confirming their potential role as indicator to clinical outcome of OS patients. Furthermore, target genes of the interested miRNAs were analyzed, which may clearly reflect the roles of miRNAs in tumor progression. The result of miRNA-target genes interactions showed that two genes were regulated by correlated miRNAs. The up-regulated *ABLIM1* (actin binding LIM protein 1) was significantly regulated by hsa-miR-373-3p and hsa-miR-302e while the down-regulated gene *CXCL5* (chemokine C-X-C motif ligand 5) was regulated by hsa-miR-3655.

It's been reported that aberrant splicing of *ABLIM1* gene was found in the skeletal muscle of patients with DM (dystrophia myotonica) [23], a kind of common form of muscular dystrophy that affects adults. In this study, *ABLIM1* was identified as one of the critical genes of OS, and regulated by hsa-miR-373-3p and hsa-miR-302e, providing us with new insights into exploring the molecular mechanism of the role of this gene played in the disease. One of the miRNAs, hsa-miR-373-3p was reported to be able to promote invasion and metastasis of lung adenocarcinoma cells. Si-

nce osteosarcoma is frequently accompanied with lung metastasis [24], we assumed that there was somehow correlation between the expression of hsa-miR-373-3p and the occurrence of OS disease, which obviously needed further research.

Another critical gene, *CXCL5*, has been reported to be an important factor in cancer biology and recent studies demonstrated that CXCL5 directly stimulates cancer cells and endothelial cells proliferation and invasion [25-28]. Prior research revealed that it can promote tumor angiogenesis in many kinds of cancers, such as, non-small cell lung carcinoma and pancreatic cancer to modulate tumor growth metastasis [29, 30]. Besides, 80% of primary lung adenocarcinomas and 65% of lung adenocarcinoma cell lines were observed tumor-specific methylation of CXCL5 [31]. However, the expression of CXCL5 and mechanisms of how CXCL5 functions in OS progress remain elusive.

In summary, we identified three markers hsa-miR-182, hsa-miR-486-5p and hsa-miR760, which may serve as the targets in the developments and treatments of OS. More importantly, two miRNA-target genes interactions were found out, providing new views on diagnosis and prognosis of OS patients.

Disclosure of conflict of interest

None.

Address correspondence to: Wengui Xie, Department of Spinal Surgery, North Medical District of Linyi People's Hospital Group, North Part of Yimeng Road, East of Linyi Party School, Linyi 276005, Shandong, China. Tel: +8615087732351; E-mail: wenguixie1@163.com

References

[1] Eyre R, Feltbower RG, Mubwandarikwa E, Eden TO and McNally RJ. Epidemiology of bone tumours in children and young adults. Pediatr Blood Cancer 2009; 53: 941-952.

[2] Mirabello L, Troisi RJ and Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. Int J Cancer 2009; 125: 229-234.

[3] Hogendoorn P, Athanasou N, Bielack S, De Alava E, Dei Tos A, Ferrari S, Gelderblom H, Grimer R, Hall KS and Hassan B. Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21: v204-v213.

[4] Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M and Winkelmann W. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J Clin Oncol 2002; 20: 776-790.

[5] Robertson J, Hollinger MJ and Wilson C. Toxicity of doxorubicin and methotrexate in osteogenic sarcoma. Br Med J 1976; 1: 23-23.

[6] Bohr VA. Rising from the RecQ-age: the role of human RecQ helicases in genome maintenance. Trends Biochem Sci 2008; 33: 609-620.

[7] Grier HE. The Ewing family of tumors: Ewing's sarcoma and primitive neuroectodermal tumors. Pediatr Clin North Am 1997; 44: 991-1004.

[8] Craft A, Cotterill S, Malcolm A, Spooner D, Grimer R, Souhami R, Imeson J and Lewis I. Ifosfamide-containing chemotherapy in Ewing's sarcoma: The Second United Kingdom Children's Cancer Study Group and the Medical Research Council Ewing's Tumor Study. J Clin Oncol 1998; 16: 3628-3633.

[9] Mangat KS, Jeys LM and Carter SR. Latest developments in limb-salvage surgery in osteosarcoma. Expert Rev Anticancer Ther 2011; 11: 205-215.

[10] Cao LL, Xie JW, Lin Y, Zheng CH, Li P, Wang JB, Lin JX, Lu J, Chen QY and Huang CM. miR-183 inhibits invasion of gastric cancer by targeting Ezrin. Int J Clin Exp Pathol 2013; 7: 5582-5594.

[11] Edgar R, Domrachev M and Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002; 30: 207-210.

[12] Kawamoto S, Matsumoto Y, Mizuno K, Okubo K and Matsubara K. Expression profiles of active genes in human and mouse livers. Gene 1996; 174: 151-158.

[13] Rocca-Serra P, Brazma A, Parkinson H, Sarkans U, Shojatalab M, Contrino S, Vilo J, Abeygunawardena N, Mukherjee G and Holloway E. ArrayExpress: a public database of gene expression data at EBI. C R Biol 2003; 326: 1075-1078.

[14] Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ and Chiu CM. miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 2011; 39: D163-9.

[15] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS and Eppig JT. Gene Ontology: tool for

the unification of biology. Nat Genet 2000; 25: 25-29.
[16] Kanehisa M and Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000; 28: 27-30.
[17] Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 2003; 4: P3.
[18] Ottaviano L, Schaefer KL, Gajewski M, Huckenbeck W, Baldus S, Rogel U, Mackintosh C, de Alava E, Myklebost O and Kresse SH. Molecular characterization of commonly used cell lines for bone tumor research: A trans-European EuroBoNet effort. Genes Chromosomes Cancer 2010; 49: 40-51.
[19] Mohseny AB, Machado I, Cai Y, Schaefer K-L, Serra M, Hogendoorn PC, Llombart-Bosch A and Cleton-Jansen AM. Functional characterization of osteosarcoma cell lines provides representative models to study the human disease. Lab Invest 2011; 91: 1195-1205.
[20] Kuijjer ML, Namløs HM, Hauben EI, Machado I, Kresse SH, Serra M, Llombart-Bosch A, Hogendoorn PC, Meza-Zepeda LA and Myklebost O. mRNA expression profiles of primary high-grade central osteosarcoma are preserved in cell lines and xenografts. BMC Medical Genomics 2011; 4: 1.
[21] Zhu J, Feng Y, Ke Z, Yang Z, Zhou J, Huang X and Wang L. Down-regulation of miR-183 promotes migration and invasion of osteosarcoma by targeting Ezrin. Am J Pathol 2012; 180: 2440-2451.
[22] Zhao H, Guo M, Zhao G, Ma Q, Ma B, Qiu X and Fan Q. miR-183 inhibits the metastasis of osteosarcoma via downregulation of the expression of Ezrin in F5M2 cells. Int J Mol Med 2012; 30: 1013-1020.
[23] Ohsawa N, Koebis M, Mitsuhashi H, Nishino I and Ishiura S. ABLIM1 splicing is abnormal in skeletal muscle of patients with DM1 and regulated by MBNL, CELF and PTBP1. Genes Cells 2015; 20: 121-134.
[24] Jian Y, Chen C, Li B and Tian X. Delocalized Claudin-1 promotes metastasis of human osteosarcoma cells. Biochem Biophys Res Commun 2015; 466: 356-361.
[25] Kuo PL, Chen YH, Chen TC, Shen KH and Hsu YL. CXCL5/ENA78 increased cell migration and epithelial-to-mesenchymal transition of hormone-independent prostate cancer by early growth response-1/snail signaling pathway. J Cell Physiol 2011; 226: 1224-1231.
[26] Miyazaki H, Patel V, Wang H, Edmunds RK, Gutkind JS and Yeudall WA. Down-regulation of CXCL5 inhibits squamous carcinogenesis. Cancer Res 2006; 66: 4279-4284.
[27] Takahashi H, Numasaki M, Lotze MT and Sasaki H. Interleukin-17 enhances bFGF-, HGF- and VEGF-induced growth of vascular endothelial cells. Immunol Lett 2005; 98: 189-193.
[28] Matsuo Y, Raimondo M, Woodward TA, Wallace MB, Gill KR, Tong Z, Burdick MD, Yang Z, Strieter RM and Hoffman RM. CXC-chemokine/CXCR2 biological axis promotes angiogenesisin vitro and in vivo in pancreatic cancer. Int J Cancer 2009; 125: 1027-1037.
[29] Arenberg DA, Keane MP, DiGiovine B, Kunkel SL, Morris SB, Xue YY, Burdick MD, Glass MC, Iannettoni MD and Strieter RM. Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. J Clin Invest 1998; 102: 465.
[30] Li A, King J, Moro A, Sugi MD, Dawson DW, Kaplan J, Li G, Lu X, Strieter RM and Burdick M. Overexpression of CXCL5 is associated with poor survival in patients with pancreatic cancer. Am J Pathol 2011; 178: 1340-1349.
[31] Tessema M, Klinge DM, Yingling CM, Do K, Van Neste L and Belinsky SA. Re-expression of CXCL14, a common target for epigenetic silencing in lung cancer, induces tumor necrosis. Oncogene 2010; 29: 5159-5170.