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Microarray expression profile of long noncoding RNAs in human osteosarcoma

Jin-ping Li [a], Li-hong Liu [a], Jie Li [b], You Chen [a], Xiao-wei Jiang [a], Yu-rong Ouyang [a], Yin-qi Liu [a], Hui Zhong [a], Hui Li [a], Tao Xiao [a,*]

[a] Department of Orthopedics, The Second Xiangya Hospital of Central South University, Changsha 410010, PR China
[b] Department of Nephrology, Zhuzhou No. 1 Hospital, Zhuzhou 412000, PR China

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ABSTRACT

Long noncoding RNAs (lncRNAs) are pervasively transcribed and have a critical role in genome regulation. Alterations in the expression of several lncRNAs have been observed in some types of cancers; however, the contributions of lncRNAs to osteosarcoma remain unknown. Here, we describe the expression profile of lncRNAs in osteosarcomas compared with paired adjacent noncancerous tissue using microarray analysis. In our study, 25,733 lncRNAs were expressed in osteosarcoma; 403 lncRNAs were consistently over-regulated and 798 lncRNAs were consistently under-regulated in all samples analyzed ($\geqslant$2.0-fold, $p < 0.05$). Quantitative real-time polymerase chain reaction (PCR) was used to validate six over-regulated and four under-regulated lncRNAs. Bioinformatic analysis (gene ontology analysis, pathway analysis and network analysis) was used for further research. Pathway analysis indicated that 32 pathways corresponded to under-regulated transcripts and that 34 pathways corresponded to over-regulated transcripts (p-value cut-off is 0.05). Our results are the first to reveal differentially expressed lncRNAs in osteosarcoma and suggest that lncRNAs may be novel candidate biomarkers for the diagnosis of osteosarcoma and potential targets for therapy.

1. Introduction

Osteosarcoma is the most common primary bone tumor in children and adolescents, accounting for approximately 15% of all bone tumors [1]; furthermore, among all primary malignant bone tumors, it is second in frequency to only multiple myeloma. Osteosarcoma is a devastating disease with poor early diagnosis and a low long-term survival rate despite significant improvements in the application of neoadjuvant chemotherapy. Unfortunately, the morbidity of osteosarcoma has been increasing by 1.4% per year for the past 25 years [2].

Genetic regulation is crucial for the occurrence of many diseases, including tumor osteogenic oncogenesis. Mutation of the p53 gene, which is the best-studied tumor suppressor gene with a regulatory function, is frequently found in osteosarcoma [3]. P53 gene therapy, which uses a transferring-modified cationic liposome, is one available treatment strategy for osteosarcoma patients [4]. The human genome comprises not only sequences encoding proteins but also a myriad of non-protein coding RNAs that may be involved in important biological processes and the regulation of molecular mechanisms [5]. Many long noncoding RNAs (lncRNAs) have been found in human and mouse using large-scale analyses of full-length cDNA sequences and other methods [6]. An increasing amount of evidence suggests that lncRNAs are involved in a variety of regulatory processes, including transcriptional regulation and epigenetic gene regulation, as well as disease [5]. lncRNAs are over 200 nucleotides in length and are separate from the other known ncRNA subsets. Previous research has focused primarily on short ncRNAs, such as microRNAs, transfer RNAs and short interfering RNAs. MicroRNAs, which are the most-studied short RNAs, play an important role in certain processes and pathways involved in development, differentiation and proliferation as well as in apoptosis and cancer [7]. Over the last decade, much research has led to considerable progress in understanding lncRNAs; however, the exact function of most lncRNAs is currently unknown. Some preliminary studies have reported that alterations in the levels of several lncRNAs have been detected in glioblastoma [8], hepatocellular carcinoma [9] and pancreatic cancer [10]. However, the biological functions of lncRNAs in osteosarcoma as well as the correlation between osteosarcoma and the expression levels of these lncRNAs remain unclear.

In this study, we profiled the expression of lncRNAs and mRNAs in nine osteosarcoma samples and their paired adjacent noncancerous samples using microarray analysis. Our results showed that the lncRNA and the mRNA expression profiles differ significantly between normal bone tissue and osteosarcoma. This finding suggests that the altered expression levels of lncRNAs may contribute to the occurrence and molecular processes of osteosarcoma and that analyzing the differences in lncRNA expression profiles may provide new methods for diagnosing and treating osteosarcoma.

* Corresponding author. Fax: +86 731 85292168.
E-mail address: xiaotaoxyl@163.com (T. Xiao).

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2. Materials and methods

2.1. Patient samples and RNA extraction

A total of 13 human primary osteosarcoma samples and their paired adjacent noncancerous tissue samples were obtained with informed consent from patients of the Central South University, XiangYa Medical College, Second XiangYa Hospital. Nine of these patient samples were randomly selected for human lncRNA microarray analysis. Our processes were in accordance with the Local Research Ethics Committee. Total RNA was extracted from 13 primary osteosarcoma samples and their paired adjacent noncancerous tissues using TRIzol reagent (Invitrogen, CA, USA). Total RNA from each sample was quantified using a NanoDrop ND-1000, and RNA integrity was assessed using standard denaturing agarose gel electrophoresis.

2.2. Microarray and data analysis

2.2.1. DNA microarray

The Human 12 × 135 k Long Non-coding RNA Array (manufactured by Roche NimbleGen, GEO: GPL11269) was a broad view that represented all long transcripts, both protein coding mRNAs and lncRNAs (long non-coding RNAs) in the human genome. More than 21,000 lncRNAs were collected from the authoritative data sources including NCBI RefSeq, UCSC, RNAdb, LncRNAs from literatures and UCRs. Sequences were selected with proprietary strategies. Repeat sequences and ncRNAs shorter than 200 bp were deleted. Each Hunan lncRNA array was composed of 60,302 distinct probes (60 mers) and each transcript was represented by 1–5 probes to improve statistical confidence. Each transcript was represented by a specific exon or splice junction probe which can identify individual transcripts accurately. The microarray analysis was performed by KangChen Bio-tech, Shanghai, PR China.

2.2.2. RNA labeling and array hybridization

Double-strand cDNA (ds-cDNA) was synthesized from 5 μg of total RNA using an Invitrogen SuperScript ds-cDNA synthesis kit in the presence of 100 pmol oligo dT primers. The ds-cDNA was cleaned and labeled in accordance with the NimbleGen Gene Expression Analysis protocol (NimbleGen Systems, Inc., USA). Briefly, ds-cDNA was incubated with 4 μg RNase A at 37 °C for 10 min and cleaned using phenol:chloroform:isoamyl alcohol followed by ice-cold absolute ethanol precipitation. The purified cDNA was quantified using NanoDrop ND-1000 and labeled with Cy3 using a NimbleGen One-Color DNA labeling kit according to the manufacturer's guidelines detailed in the Gene Expression Analysis protocol (NimbleGen Systems, Inc., Madison, WI, USA). One microgram of ds-cDNA was incubated for 10 min at 98 °C with 1 OD of Cy3–9 mer primer. In addition, 100 pmol of deoxynucleoside triphosphates and 100 U of the Klenow fragment (New England Biolabs, USA) were added, and the mix was incubated at 37 °C for 2 h. The reaction was stopped by adding 0.1 volume of 0.5 M EDTA, and the labeled ds-cDNA was purified by isopropanol/ethanol precipitation. Microarrays were hybridized at 42 °C during 16–20 h with 4 μg of Cy3-labeled ds-cDNA in NimbleGen hybridization buffer/hybridization component A in a hybridization chamber (Hybridization System-NimbleGen Systems, Inc., Madison, WI, USA). Following hybridization, washing was performed using the NimbleGen Wash Buffer kit (NimbleGen Systems, Inc., Madison, WI, USA). After being washed in an ozone-free environment, the slides were scanned using an Axon GenePix 4000B microarray scanner [8]. The microarray analysis was performed by KangChen Bio-tech, Shanghai, PR China.

2.3. Data analysis

Slides were scanned at 5 μm/pixel resolution using an Axon GenePix 4000B scanner (Molecular Devices Corporation) piloted by GenePix Pro 6.0 software (Axon). Scanned images (TIFF format) were then imported into NimbleScan software (version 2.5) for grid alignment and expression data analysis. Expression data were normalized through quantile normalization and the Robust Multichip Average (RMA) algorithm included in the NimbleScan software. The probe level files and mRNA level files were generated after normalization. All gene level files were imported into Agilent GeneSpring GX software (version 11.5.1) and normalized by the quantile method; then, Combat software was used to adjust the normalized intensity to remove batch effects. Significantly differentially expressed lncRNAs and mRNAs were identified through Volcano Plot filtering. Hierarchical clustering was performed using Agilent GeneSpring GX software (version 11.5.1) [8]. The analysis was performed by KangChen Biotech., Shanghai, PR China.

2.3.1. Functional group analysis

The Gene Ontology (GO) project provides a controlled vocabulary to describe gene and gene product attributes in any organism (http://www.geneontology.org). The ontology covers three domains: biological process, cellular component and molecular function. Fisher's exact test was used to determine whether the overlap between the differentially expressed (DE) list and the GO annotation list is greater than that expected by chance. The *p*-value denotes the significance of the GO term enrichment in the DE genes FDR stranded for the false discovery rate .The lower the *p*-value is, the more significant the GO term (a *p*-value $\leqslant 0.05$ is recommended). Pathway analysis is a functional analysis that maps genes to KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (http://www.genome.jp/kegg/). The *p*-value (EASE-score, Fisher *p* value or Hypergeometric *p* value) denotes the significance of the pathway correlated to the conditions. The lower the *p*-value is, the more significant the correlation. (The recommend *p*-value cut-off is 0.05.)

2.3.2. The co-expression network

To investigate the relation between lncRNAs and their coding genes, we constructed a coding-noncoding gene co-expression network using 12 lncRNAs and their coding genes as follows. (i) The data were preprocessed by using the median gene expression value of all transcripts expressed from the same coding gene, without special treatment of the lncRNA expression value. (ii) We then screened the data for differentially expressed lncRNAs and mRNAs and removed these data from the dataset. (iii) We calculated the Pearson correlation coefficient and used the *R* value to calculate the correlation coefficient of the PCC between lncRNA coding genes (only lncRNA-coding PCC, not including lncRNA–lncRNA or coding–coding PCC). (iv) Finally, we screened based on Pearson correlation coefficient by selecting PCC$\geq$0.95 as meaningful and drawing the co-expression network using Cytoscape. In the network, a pink node represents the coding gene, a red node represents an over-regulated lncRNA, and a green node represents a under-regulated lncRNA. A solid line indicates a positive correlation, and a dashed line indicates a negative correlation.

2.3.3. Quantitative real-time PCR

The total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA) and then reverse transcribed using PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian,

China) according to the manufacturer's recommendations. The expression of over-regulated lncRNAs (ASLNC21868, ASLNC22124, ASLNC23844, ASLNC24587, BE503655, BC050642) and under-regulated lncRNAs (ASLNC00339, ASLNC11435, ASLNC13387, ASLNC18814) in all patients included in this study was measured by real-time PCR using SYBRGreen assays (TaKaRa, Dalian, China), and GAPDH was used as an internal control. The primers are listed in Supplementary Table S1. For quantitative results, the expression of each lncRNA was represented as fold change using $2^{-\Delta\Delta Ct}$ methods [11]. The lncRNA expression differences between human primary osteosarcomas and their paired adjacent noncancerous tissues were analyzed using Student's *t*-test with SPSS (Version 16.0 SPSS Lnc). A value of $p < 0.05$ was considered significant.

3. Results

3.1. Differentially expressed lncRNAs

In the lncRNA expression profiling data, we found a total of 25,733 lncRNAs expressed in osteosarcoma using microarray analysis (Fig. 1A, Supplementary Table S2). Using these data, we compared the lncRNA expression levels between nine human primary osteosarcomas and their paired adjacent noncancerous samples and identified an average of 3528 over-regulated lncRNAs (range from 2723 to 4537) and 3958 under-regulated lncRNAs (range 3469–4368) that were significantly differentially expressed ($\geqslant$2.0-fold) (Fig. 2A and B). Among these lncRNAs, 403 were consistently over-regulated, and 798 lncRNAs were consistently

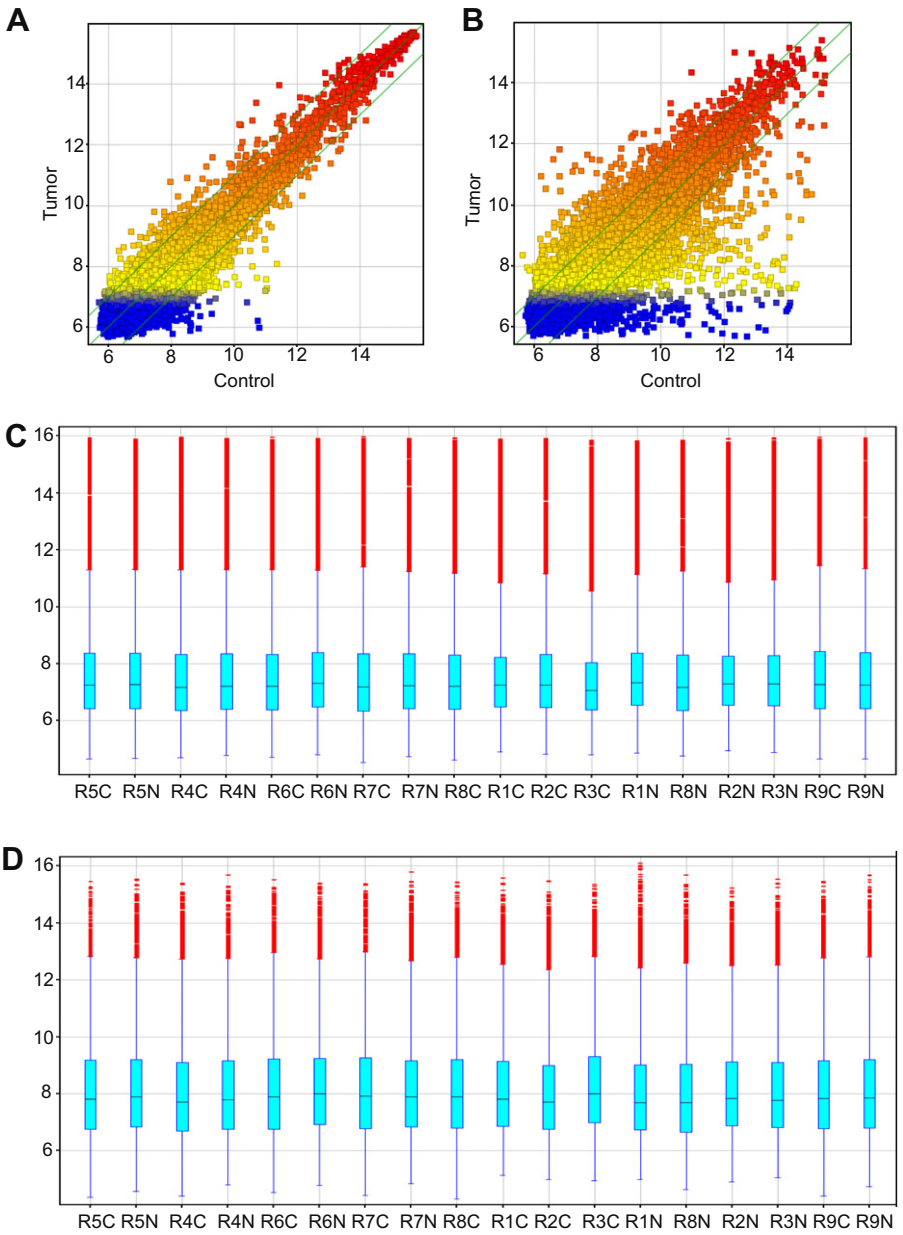

Fig. 1. The expression profiles of lncRNAs and mRNAs were compared between osteosarcomas and paired adjacent noncancerous samples. The box plot is a convenient way to quickly visualize the distributions of a dataset for the lncRNAs (A) and the mRNAs (B) profiles. After normalization, the distributions of log2 ratios among nine samples are nearly the same. The scatter plot is a visualization method used for assessing the lncRNA (C) and mRNA (D) expression variations between osteosarcomas and paired adjacent noncancerous samples. The values of the *X* and *Y* axes in the scatter plot are the averaged normalized signal values of the group (log2 scaled). The green lines are fold change lines (the default fold change given is 2.0).

A

Down-regulated lncRNAs		Up-regulated lncRNAs	
lncRNAs	log2 fold change(T/N)	lncRNAs	log2 fold change(T/N)
ASLNC02724	7.2276354	ASLNC05129	27.386826
ASLNC10032	6.5696545	ASLNC04683	22.388136
ASLNC23844	5.88796	ASLNC21792	13.600891
ASLNC21868	5.7517543	ASLNC20271	13.130057
ASLNC10044	5.533821	ASLNC11435	11.647422
ASLNC09173	5.4589977	ASLNC00560	10.517859
ASLNC09280	5.432108	ASLNC18261	9.553265
ASLNC24079	5.217446	ASLNC22440	8.431529
ASLNC14632	4.972277	ASLNC10918	7.4500036
ASLNC04893	4.7651844	ASLNC24310	7.0071945
ASLNC06949	4.6425366	ASLNC08248	6.6065974
AK000168	4.594313	ASLNC12957	6.3973017
ASLNC08413	4.4864907	ASLNC03816	5.8261676
ASLNC13907	4.332455	DN996211	5.8198376
X14355	4.1451435	ASLNC21283	5.673939
ASLNC02419	3.9984086	AK299815	5.4372163
EC581812	3.9626284	ASLNC03700	5.398673
ASLNC18342	3.9517286	AM392923	5.239583
EC506875	3.8812556	ASLNC07956	5.0942802
ASLNC04430	3.7936206	ASLNC23339	5.074055
ASLNC16219	3.7370079	ASLNC03665	4.931716

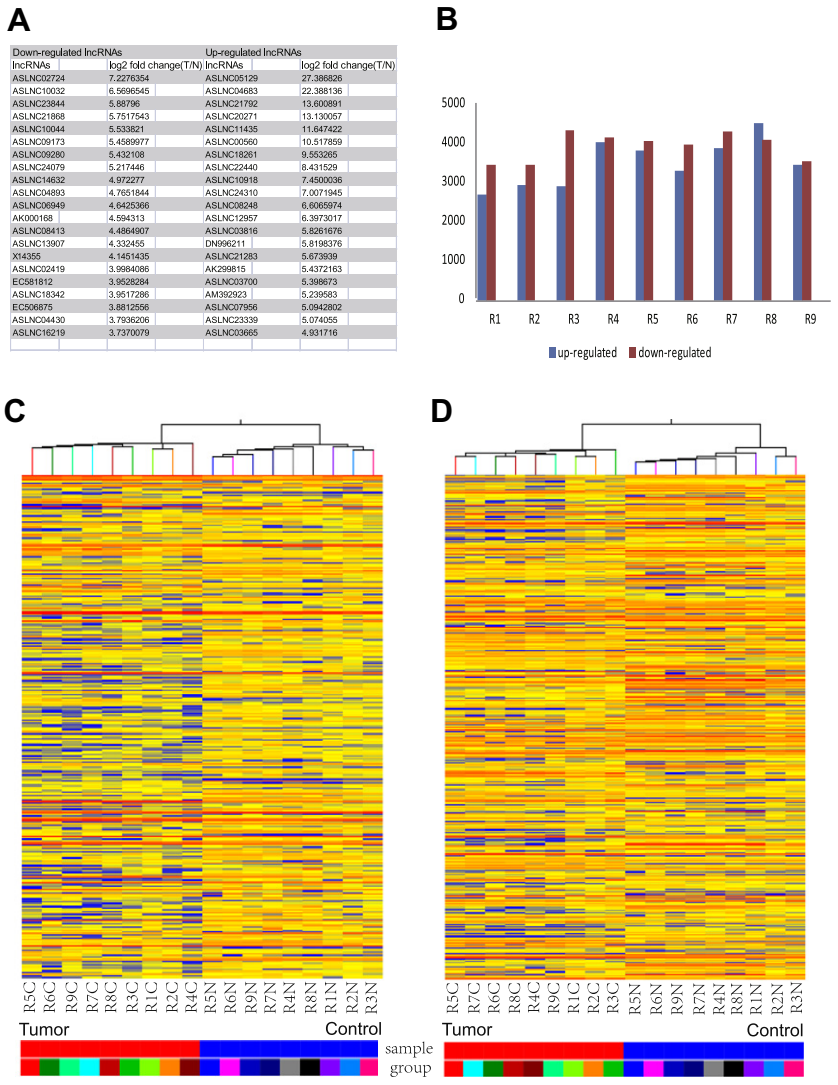

Fig. 2. The differentially expressed profiles in osteosarcomas were compared with paired adjacent noncancerous samples. (A) A subset of the differentially expressed lncRNAs detected using microarray analysis was selected and summarized (sample, $N = 9$); (B) the counts of over-regulated and under-regulated lncRNAs varied across the nine samples; (C) differentially expressed lncRNAs and (D) differentially expressed mRNAs were analyzed using hierarchical clustering. Hierarchical clustering analysis arranges samples into groups based on their expression level, which allows us to hypothesize the relationships among samples. "Red" indicates high relative expression, and "Blue" indicates low relative expression. It was revealed that under-regulated lncRNAs were more common than the over-regulated ones, while there were similar numbers of under-regulated mRNAs and over-regulated ones. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

under-regulated (Supplementary Table S3). We used hierarchical clustering analysis to arrange samples into groups based on their expression levels, which allowed us to hypothesize the relationships among samples. The dendrogram shows the relationships among the lncRNA expression patterns between samples (Fig. 2C). ASLNC02724 (fold change: 7.2276354) was the most over-regulated lncRNA, and ASLNC05129 (fold change: 27.386826) was the most under-regulated lncRNA. Over-regulated lncRNAs were less common than under-regulated lncRNAs in our microarray data.

3.2. Differentially expressed mRNAs

From the mRNA expression profiling data, a total of 16,352 mRNAs were identified in the osteosarcoma samples through microarray analysis (Fig. 1B, Supplementary Table S4). Among the nine human primary osteosarcomas and their paired adjacent noncancerous samples, an average of 2604 over-regulated mRNAs (range from 1394 to 3710) and 2344 under-regulated mRNAs (range 1513–2867) were significantly differentially expressed ($\geqslant$2.0-fold). In the osteosarcoma samples, 986 mRNAs were consistently over-regulated, and 933 mRNAs were consistently under-regulated (Supplementary Table S5). The clustering analysis also showed the relationships among the mRNA expression patterns that were present in the samples (Fig. 2D).

3.3. GO analysis and pathway analysis

GO analysis was performed to determine the gene and gene product enrichment in biological processes, cellular components and molecular functions. Fisher's exact test was used to determine whether the overlap between the differentially expressed gene list and the GO annotation list was greater than that expected by chance (a p-value $\leqslant$0.05 is recommended, Fig. 3A). We found that the highest enriched GOs targeted by under-regulated transcripts were muscle system process (ontology: biological process), contractile fiber (ontology: cellular component) and structural constituent of muscle (ontology: molecular function) and that the highest

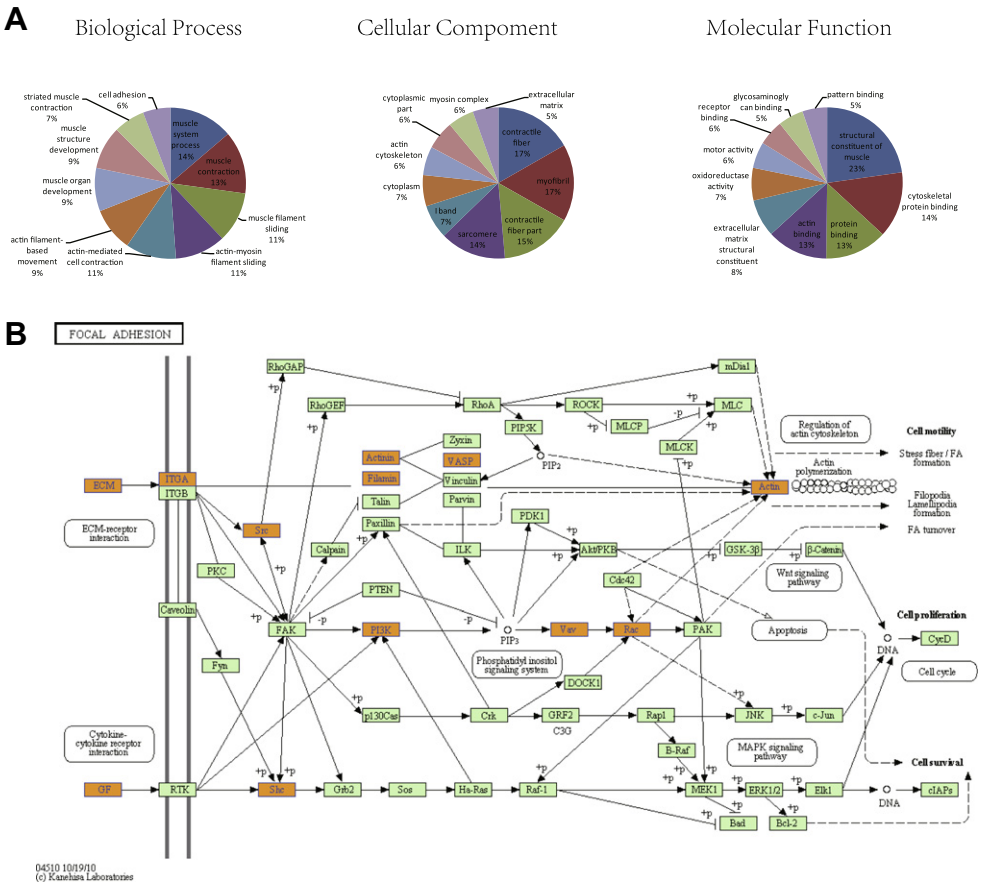

Fig. 3. Bioinformatic analysis of the differentially expressed profiles. (A) The Gene Ontology enrichment analysis provides a controlled vocabulary to describe differentially expressed transcript attributes in all organisms. The ontology covers three domains: biological process, cellular component and molecular function. (p-value $\leq$0.05 is recommended); (B) pathway analysis is a functional analysis that maps genes to KEGG pathways. The "Focal Adhesion" signal pathway shows modulation in tumor signatures and is associated with an outcome of osteosarcoma.

enriched GOs targeted by the over-regulated transcripts were cell adhesion (ontology: biological process), extracellular matrix (ontology: cellular component) and protein binding (ontology: molecular function) (Supplementary Table S6). Pathway analysis indicated that 32 pathways corresponded to under-regulated transcripts and that the most enriched network was "Hypertrophic cardiomyopathy-Homo sapiens (human)" composed of 24 targeted genes. Furthermore, this analysis showed that 34 pathways corresponded to over-regulated transcripts and that the most enriched network was "ECM-receptor interaction-Homo sapiens (human)" composed of 22 targeted genes (Supplementary Table S7, the recommend p-value cut-off is 0.05). One of these pathways, the gene category "Focal adhesion", has been reported to be involved in the development of osteosarcoma [12] (Fig. 3B).

3.4. Construction of the co-expression network

We constructed a coding-noncoding gene co-expression network that included the differentially expressed lncRNAs and targeted coding genes. The lncRNAs and coding genes that had Pearson correlation coefficients equal to or greater than 0.95 were chosen to draw the network using Cytoscape. The result showed that the co-expression network was composed of 239 network nodes and 393 connections between 12 lncRNAs and 227 coding genes. Within this co-expression network, 327 pairs presented as positive, and 66 pairs presented as negative. This co-expression network indicated that one lncRNA could target 122 coding genes at most and that one coding gene could correlate with three lncRNAs at most (Supplementary Table S8).

3.5. Real time quantitative PCR validation

We randomly selected six over-regulated lncRNAs (ASLNC21868, ASLNC22124, ASLNC23844, ASLNC24587,BE503655, BC050642) and four under-regulated lncRNAs (ASLNC00339, ASLNC11435, ASLNC13387, ASLNC18814) from the differentially expressed lncRNAs. To identify the expression levels of the altered lncRNAs in osteosarcoma patients, two sets of bone tissues were probed for the expression of these lncRNAs using quantitative real-time PCR (qPCR). The results demonstrated that ASLNC21868, ASLNC22124, ASLNC23844, ASLNC24587, BE503655 and BC050642 were over-regulated and that ASLNC00339, ASLNC11435, ASLNC13387 and ASLNC18814 were under-regulated in the osteosarcoma samples compared with normal samples. The qPCR results and microarray data are consistent ($p < 0.05$, Fig. 4A and B).

4. Discussion

Previous studies have shown that lncRNAs have a significant role in genome regulation, the expression of which is involved in a variety of human diseases, including cancers [13,14]. Of all the functions of lncRNAs, the most important one is their involvement in tumorigenesis, which has been shown in live cancer cells [15]. The p53 gene can be regulated by the lncRNA MEG3 [16], and alterations in p53 are common in osteosarcoma [17]; therefore, there may be a connection between the lncRNA MEG3 and the development of osteosarcoma. Our team has already found that miR-125b can suppress human osteosarcoma cell proliferation and migration through the down-regulation of a signal transducer and an

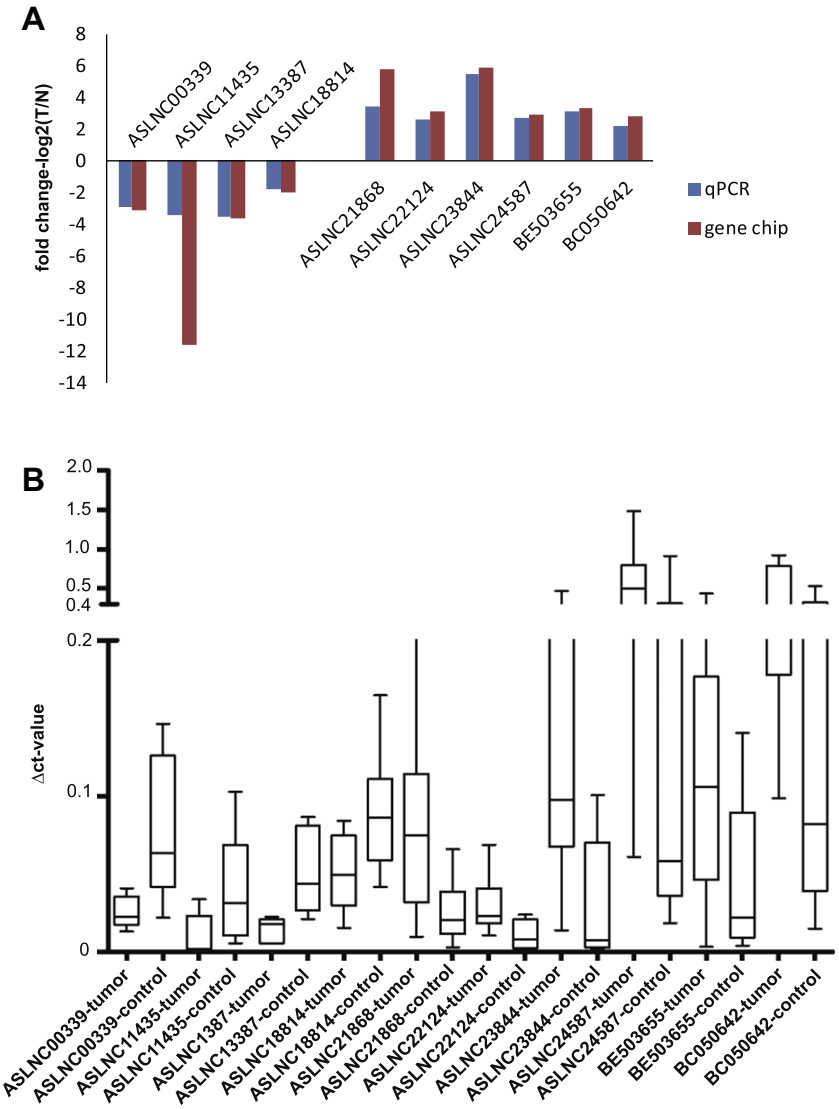

Fig. 4. The differential expression of lncRNAs was validated by quantitative real-time PCR. (A) Comparison between microarray and quantitative real-time PCR results. Ten differentially expressed lncRNAs were validated by qPCR. The heights of the columns in the chart represent the log-transformed median fold changes (*T*/*N*) in expression across nine samples ($p \leqq 0.05$). The qPCR results and microarray data are consistent. (B) Distributions of the lncRNA expression levels ($p \leqq 0.05$). Ten differentially expressed lncRNAs were validated by qPCR in 13 human osteosarcomas and paired adjacent noncancerous samples.

activator of transcription 3 (STAT3) [18]. However, the pathogenesis of osteosarcoma remains unclear, and there are no reports on lncRNA expression profiles in osteosarcoma.

In the present study, we investigated the lncRNA expression profiles of osteosarcoma using microarray analysis and found that the lncRNA expression levels were altered compared to adjacent noncancerous tissues. This paper is the first to report the expression of lncRNAs in osteosarcoma. We analyzed nine human primary osteosarcomas and their paired adjacent noncancerous samples using microarray analysis and selected a small number of lncRNAs for validation by quantitative real-time PCR in 13 human primary osteosarcoma samples and their paired adjacent noncancerous tissue samples. From the microarray expression profiles, we found that 25,733 lncRNAs were expressed; 403 over-regulated lncRNAs and 798 under-regulated lncRNAs were significantly differentially expressed ($\geqslant$2.0-fold) in all osteosarcoma samples. Ten natural antisense lncRNAs were evaluated by quantitative real-time PCR to validate the consistency. Furthermore, we utilized Gene Ontology (GO) analysis and pathway analysis and constructed a co-expression network to study preliminarily the biological functions of these lncRNAs in the development of osteosarcoma.

For osteosarcoma, genetic mutations in development genes were common, as observed in other cancers. A under-regulated lncRNA, ASLNC18814, was found to be located near murine double minute 2 (MDM2), which interacts with p53 and selectively activates the p53 pathway to promote proliferation and suppress apoptosis in osteosarcoma cells [19]. A natural antisense relationship between the under-regulated lncRNAs ASLNC18814 and MDM2 may help us learn more about osteosarcoma and lncRNAs at the transcriptional level. An over-regulated lncRNA BE050604 was found to be associated with c-myc promoter-binding protein-1 in our profiles. The expression of c-myc protein was correlated with prognosis in osteosarcoma patients, and it could be used to predict the progression and prognosis of osteosarcoma [20]. To understand the functions of lncRNAs further, we applied pathway analysis to study the differentially expressed lncRNAs and found that 32 pathways corresponded to under-regulated transcripts and 34 pathways corresponded to over-regulated transcripts. One of those pathways, "Focal adhesion" signal pathway showed modulation in tumor signatures, such as transforming growth factors in osteosarcoma, and the pathway was associated with outcomes for osteosarcoma [12]. In our study, 68 pathways

corresponding to differentially expressed transcripts were found, and some or all may be involved in the pathogenesis of osteosarcoma.

In conclusion, we report for the first time that lncRNAs are differentially expressed in osteosarcoma when compared with paired adjacent noncancerous tissues. Additionally, our study revealed that ASLNC18814 is located near MDM2 and that BE050604 is associated with c-myc protein. It is suggested that lncRNAs may exert their functions through interactions with coding transcripts and proteins in osteosarcoma. Additional signal pathways that use lncRNAs to regulate biological activities will be found. An investigation into the connections between signal pathways and lncRNAs is critical for developing novel strategies for the early diagnosis and treatment of osteosarcoma. Further work is needed to understand the molecular mechanisms and biological functions of lncRNAs in osteosarcoma.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.02.083.

References

[1] P. Picci, Osteosarcoma (osteogenic sarcoma), Orphanet. J. Rare Dis. 2 (2007) 6.
[2] J.S. Caudill, C.A. Arndt, Diagnosis and management of bone malignancy in adolescence, Adolesc. Med. State Art. Rev. 18 (2007) 62–78.
[3] B. Wadayama, J. Toguchida, T. Yamaguchi, M.S. Sasaki, T. Yamamuro, P53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas, Br. J. Cancer 68 (1993) 1134–1139.
[4] M. Nakase, M. Inui, K. Okumura, T. Kamei, S. Nakamura, T. Tagawa, P53 gene therapy of human osteosarcoma using a transferrin-modified cationic liposome, Mol. Cancer Ther. 4 (2005) 625–631.
[5] C.P. Ponting, P.L. Oliver, W. Reik, Evolution and functions of long noncoding RNAs, Cell 136 (2009) 629–641.
[6] M.E. Dinger, K.C. Pang, T.R. Mercer, M.L. Crowe, S.M. Grimmond, J.S. Mattick, NRED: a database of long noncoding RNA expression, Nucleic Acids Res. 37 (2009) D122–D126.
[7] S. Griffiths-Jones, H.K. Saini, S. van Dongen, A.J. Enright, MiRBase: tools for microRNA genomics, Nucleic Acids Res. 36 (2008) D154–D158.
[8] L. Han, K. Zhang, Z. Shi, J. Zhang, J. Zhu, S. Zhu, A. Zhang, Z. Jia, G. Wang, S. Yu, P. Pu, L. Dong, C. Kang, LncRNA profile of glioblastoma reveals the potential role of lncRNAs in contributing to glioblastoma pathogenesis, Int. J. Oncol. 40 (2012) 2004–2012.
[9] F. Yang, L. Zhang, X.S. Huo, J.H. Yuan, D. Xu, S.X. Yuan, N. Zhu, W.P. Zhou, G.S. Yang, Y.Z. Wang, J.L. Shang, C.F. Gao, F.R. Zhang, F. Wang, S.H. Sun, Long noncoding RNA high expressed in hepatocellular carcinoma (lncRNA-HEIH) facilitates tumor growth through enhancer of zeste homolog 2, Hepatology 54 (2011) 1679–1689.
[10] A.C. Tahira, M.S. Kubrusly, M.F. Faria, B. Dazzani, R.S. Fonseca, V. Maracaja-Coutinho, S. Verjovski-Almeida, M.C. Machado, E.M. Reis, Long noncoding intronic RNAs are differentially expressed in primary and metastatic pancreatic cancer, Mol. Cancer 10 (2011) 141.
[11] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) e45.
[12] L.E. O'Donoghue, A.A. Ptitsyn, D.A. Kamstock, J. Siebert, R.S. Thomas, D.L. Duval, Expression profiling in canine osteosarcoma: identification of biomarkers and pathways associated with outcome, BMC Cancer 10 (2010) 506.
[13] L.-L. Chen, G.G. Carmichael, Decoding the function of nuclear long non-coding RNAs, Curr. Opin. Cell Biol. 22 (2010) 357–364.
[14] M.E. Dinger, A.S. Arefin, R. Berretta, P. Moscato, The hippocampal expression of 31 noncoding Rnas is upregulated in Alzheimer's disease patients and three positively correlate with MMSE results and other biomarkers of disease progression, Alzheimer's and Dementia 6 (2010) e47–e48.
[15] J.Y. Wang, X.F. Liu, H.C. Wu, P.H. Ni, Z.D. Gu, Y.X. Qiao, N. Chen, F.Y. Sun, Q.S. Fan, CREB up-regulates long noncoding RNA, HULC expression through interaction with microRNA-372 in liver cancer, Nucleic Acids Res. 38 (2010) 5366–5383.
[16] L. Lipovich, R. Johnson, C.Y. Lin, MacroRNA underdogs in a microRNA world: Evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA, Biochim. Biophys. Acta 1799 (2010) 597–615.
[17] L.L. Wang, Biology of osteogenic sarcoma, Cancer J. 11 (2005) 294–305.
[18] L.H. Liu, H. Li, J.P. Li, H. Zhong, H.C. Zhang, J. Chen, T. Xiao, MiR-125b suppresses the proliferation and migration of osteosarcoma cells through down-regulation of STAT3, Biochem. Biophys. Res. Commun. 416 (2011) 31–38.
[19] B. Wang, L. Fang, H. Zhao, T. Xiang, D. Wang, MDM2 inhibitor Nutlin-3a suppresses proliferation and promotes apoptosis in osteosarcoma cells, Acta Biochim. Biophys. Sin. 44 (2012) 685–691.
[20] X. Wu, Z.D. Cai, L.M. Lou, Y.B. Zhu, Expressions of p53, c-MYC, BCL-2 and apoptotic index in human osteosarcoma and their correlations with prognosis of patients, Cancer Epidemiol. 36 (2012) 212–216.