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Weighted gene correlation network analysis identifies the critical long non-coding RNAs participate in the progression of osteosarcoma

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Abstract. This study aimed to identify more biomarkers associated with osteosarcoma progression *via* lncRNA-mRNA co-expression network. Dataset GSE99671 was downloaded from GEO database. The mRNAs and lncRNAs that were differentially expressed between tumor and normal samples were screened out. Functional enrichment analysis of differentially expressed mRNAs was carried out, followed by weighted gene correlation network analysis (WGCNA). Based on the lncRNAs and mRNAs, a lncRNA-mRNA co-expression network was constructed. Total 703 mRNAs and 7 lncRNAs were differentially expressed between tumor and normal tissues. The mRNAs were significantly enriched in functions associated with inflammatory response as well as autoimmune thyroid disease and ribosome pathways. WGCNA revealed that ME2 module had a high correlation with tumor, and *ST3GAL4*, *UCK2*, *PSAT1* etc. had higher connectivity degrees in this module. lncRNA-mRNA co-expression network showed that 12 mRNAs, such as *PEMT*, *COL10A1* and *GSTA1*, were synergistically expressed with lncRNA TTTY14. Inflammatory response and ribosome synthesis may play important role in osteosarcoma progression. lncRNA TTTY14 may affect the development of osteosarcoma by cooperative expression with *PEMT*, *COL10A1*, *GSTA1*, etc. *ST3GAL4*, *UCK2*, *PSAT1* as well as TTTY14 may serve as key biomarkers in osteosarcoma.

Key words: Osteosarcoma — Weighted gene correlation network analysis — lncRNA — Co-expression network

Abbreviations: *COL10A1*, collagen type X alpha 1 chain; *CXCL12*, C-X-C motif chemokine ligand 12; *CXCR1*, C-X-C motif chemokine receptor 1; GSTA1, glutathione S-transferase alpha 1; MPO, myeloperoxidase; NES, normalized enrichment score; *PEMT*, phosphatidylethanolamine N-methyltransferase; *TNFRSF4*, TNF receptor superfamily member 4; WGCNA, weighted gene correlation network analysis.

Introduction

Osteosarcoma is a primary malignant bone tumor that is characterized by mesenchymal derived spindle cells depositing immature osteoid matrix (Campanacci 2013). About 56% of osteosarcomas arise in the long bones of the lower limb, with the upper limb (10%) and pelvis (9%) being the next most affected sites (Whelan et al. 2012). This tumor most commonly influences children, adolescents and young adults (Isakoff et al. 2015). Patients with osteosarcoma most often complain about swelling and pain (Picci 2007). Osteosarcoma is an aggressive tumor, and approximately 80% to 90% of patients have detectable metastases at presentation (Luetke et al. 2014). These patients have poor prognosis with long-term survival rate of only 10–30% (Meyers 2009). However, the exact pathogenesis of osteosarcoma is not yet determined. Identification of new candidate molecules is crucial for improving the clinical outcomes of patients with this tumor.

Recently, high-throughput sequencing technologies have been widely used in osteosarcoma studies (Siddiqui et al. 2006; Yang et al. 2014). With this method, many genes were

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found to be differentially expressed between osteosarcoma sample and normal bone, such as *GJA1*, *COL1A2*, *COL5A2*, *MMP14*, *TGFβ* and *RUNX2* (Wu et al. 2014). Ho et al. (2017) performed whole transcriptome analysis of osteosarcoma and normal bone samples, and identified 5365 differentially expressed genes between osteosarcoma and normal bone tissues, including *ABCA10, BTNL9*, *COL11A1*, and *MMP14*. Additionally, *via* functional enrichment analysis, they found that extracellular matrix degradation may be an important mechanism of osteosarcoma progression. They deposited the dataset in GEO with accession number of GSE99671. However, they only analyzed the mRNA data whereas the long noncoding RNA (lncRNA) data generated by whole transcriptome sequencing were not analyzed. lncRNAs are a group of RNA transcripts of over 200 nucleotides in length with no or little protein-coding capacity (Wang and Chang 2011). Recent studies have revealed that lncRNAs could act as tumor suppressors or oncogenes to play important roles in carcinogenesis (Khandelwal et al. 2015; Cui et al. 2016). Importantly, lncRNAs can regulate gene expression through diverse mechanisms including lncRNA-mRNA interaction (Ernst and Morton 2013). The lncRNAs involved in the development of osteosarcoma are still warranted investigation.

In recent years, vast RNA-seq data were generated because of the rapid development on the sequencing technology. The exponential increase of these data has stimulated the development of an ever-increasing number of bioinformatics tools (Canzoneri et al. 2019). Considerable papers re-analyzed the public available datasets and investigated the molecular mechanism of disease at the molecular level (Shen et al. 2019; Zhang et al. 2019; Sang et al. 2020; Zhou et al. 2020). These discoveries have showed great significance in revealing the molecular mechanism of disease pathogenesis and remarkably improved the early diagnosis and prognosis of disease. Thus, in this study, we downloaded this dataset from GEO database to reanalyze the dataset of GSE99671. By reannotation of the genes, the mRNAs and lncRNAs that were differentially expressed between tumor and normal samples were screened out. Based on the lncRNAs and mRNAs, we constructed the lncRNA-mRNA co-expression network to explore their regulatory relations, which may help to understand the potential mechanisms of this tumor.

Methods

Data acquisition and lncRNA reannotation

The dataset GSE99671 (GPL20148) was downloaded from the GEO database, which was obtained from the whole transcriptome sequencing of 18 pairs of tumor and normal bone tissue cells samples. According to the gtf gene annotation file provided by GENCODE database (Harrow et al. 2012) (Release 26, grch38.p10), the gene with annotation information of "protein coding" was retained as mRNA, and the gene with annotation information of "lncRNA" was retained as lncRNA.

Differential expression analysis

The data matrix was normalized using betaqn method in R. Based on the normalized dataset, we used limma package (Ritchie et al. 2015) (version 3.10.3) to analyze the differences between tumor and normal samples. The *p* values of all the genes were subjected to multiple test adjustment with Benjamini and Hochberg method, obtaining the adjusted *p* value (adj.*p*.value). The genes with adj.*p*.value < 0.05 and |log fold change (FC)| > 1 were considered as differentially expressed genes.

Functional and pathway enrichment analyses of differentially expressed mRNAs

The enrichment analysis tool DAVID (Huang da et al. 2009) (version 6.7, https://david-d.ncifcrf.gov/) was used to analyze the functional annotations of differentially expressed mRNAs, and the results were visualized using GOplot (Walter et al. 2015). Additionally, based on KEGG database (Kanehisa et al. 2000), KEGG pathway analysis was conducted using gene set enrichment analysis (GSEA, version 3.0) (Damian et al. 2004). The enrichment results with adj.*p*.value < 0.05 were screened as significant results. Normalized enrichment score (NES) that reflects the enrichment of the pathway was used to identify the activated pathways (NES > 0) and suppressed pathways (NES < 0).

Weighted gene correlation network analysis (WGCNA)

WGCNA is a systematic biological method to describe the gene correlation patterns among different samples, which can be used to identify gene sets with highly coordinated variation and identify candidate biomarker genes according to the endogeneity of gene sets and the correlation between gene sets and clinical phenotypes. Here, we used the R package of WGCNA (version 1.61) to calculate the functional set of various weighted association analysis, construct the co-expression network, identify genes and gene clusters, and calculate the topological characteristics. Briefly, the expression correlation between two genes was calculated, then the adjacency function was defined, and finally the module was divided. In addition, we calculated the correlation between gene module and clinical phenotype, and identified the modules related to traits. In the process of network construction, the correlation attribute corType was set as Pearson's correlation coefficient, and the minimum number of module genes was set as 30. For the WGCNA gene co-expression network, edges (gene pairs) with weight value more than 0.05 were selected.

lncRNA-mRNA co-expression network

For differentially expressed lncRNAs and differentially expressed mRNAs, Pearson's correlation coefficients between them were calculated using the corr.test method of R package psych (Jason 2013) (parameter ci = F, adjust = "BH"). The Benjamini and Hochberg method was used for multiple test adjustment. The gene pairs with $|r| \geq 0.6$ and adj.*p*.value < 0.05 were screened. Cytoscape software (version 3.7.0) was used to construct the co-expression network.

Results

Differential expression analysis

According to GENCODE annotation results, GSE99671 contained 23,099 genes, including 17,180 mRNAs and 104 lncRNAs. After analysis, there were 710 differentially expressed genes (241 upregulated and 469 down-regulated) (Fig. 1A). Among these genes, there were 181 upregulated mRNAs, 2 upregulated lncRNAs, 378 downregulated mRNAs and 5 downregulated lncRNAs. The top10 up- and down-regulated genes are shown in Figure 1B. The differentially expressed lncRNAs included *TTTY22, DKFZP434K028, DSCR9, RNU11, FAM138E, TERC* and *TTTY14*.

GO enrichment analysis

GO enrichment analysis of the differentially expressed mRNAs showed that a total of 6 biological process terms, 15 cellular component terms and 8 molecular function terms were significantly enriched (adj.*p*.value < 0.05). The top 5 biological process terms, cellular component terms and molecular function terms according to adj.*p*.value sorting are shown in Figure 2. In terms of the biological process terms, oxygen transport (n = 8, adj.*p*.value = 2.10×10^{-4}), defense response to bacterium (n = 9, adj.*p*.value = 2.86×10^{-4}), inflammatory response (n = 29, adj.*p*.value = 0.00576), response to lipopolysaccharide (n = 17, adj.*p*.value = 0.0149), chronic inflammatory response (n = 5, adj.*p*.value = 0.0359), and defense response to fungus (n = 7, adj.*p*.value = 0.0395) were significantly enriched. In terms of cellular component, the expressed proteins were mainly located on extracellular region (n =116, adj.*p*.value = 4.56×10^{-17}), extracellular space (n =100, adj.*p*.value = 1.93×10^{-15}), plasma membrane (n =176, adj.*p*.value = 6.28×10^{-6}), hemoglobin complex (n = 8, adj.*p*.value = 1.54×10^{-6}), etc., indicating that these genes play an important role in the communication and interaction between cells. From the perspective of molecular function, the differentially expressed mRNAs mainly possess oxygen transporter activity (n = 8, adj.*p*.value = 3.05×10^{-5}), oxygen binding (n =10, adj.*p*.value = 2.18×10^{-3}), heparin binding (n = 17, adj.*p*.value = 0.00348), and were related to the activities of peroxidase (n = 6, adj.*p*.value = 0.0450) and serine endopeptidase (n =19, adj.*p*.value = 0.0481).

Pathway enrichment analysis

According to the pathway enrichment analysis by GSEA, 7 significantly enriched pathways were identified, including 3 activated pathways (NES > 0) and 4 suppressed pathways (NES < 0) (Fig. 3A). KEGG ribosome had the maximum NES among the activated pathways (Fig. 3B). KEGG autoimmune thyroid disease had the minimum NES among the sup-

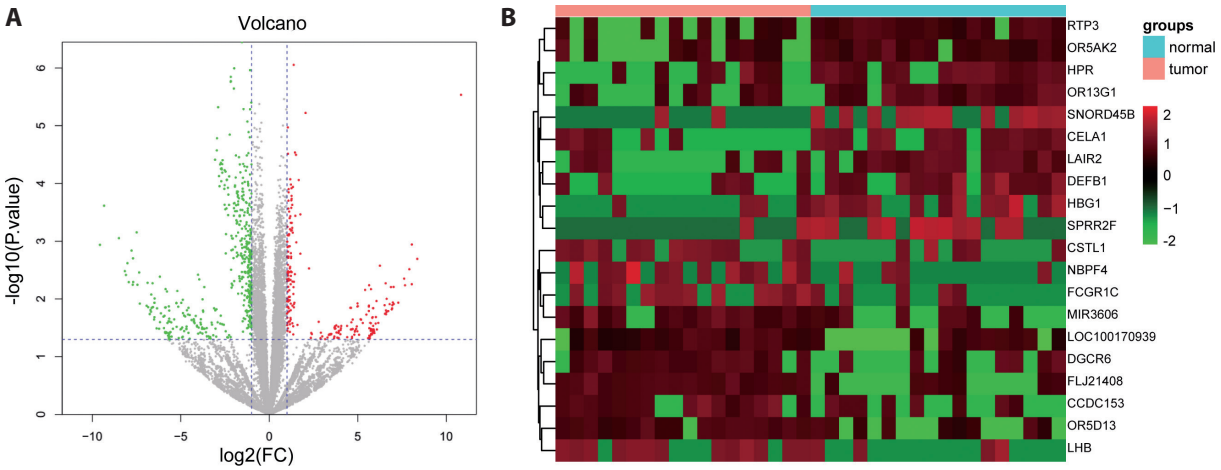

Figure 1. A. Volcano plot of differentially expressed genes. Thresholds were set as |log2(FC)| > 1 and *p* value < 0.05. Red represents upregulated gene and green represents downregulated gene. **B.** Heatmap of top10 up- and downregulated genes (in ascending order according to *p* value).

pressed pathways (Fig. 3C). Abnormalities in this pathway suggested that osteosarcoma had an impact on the thyroid immune system, leading to a decline in immune function. Additionally, cytosolic DNA sensing pathway and adipocytokine signaling pathway were also significantly suppressed, indicating that cellular interaction and immune response were abnormal, which may be correlated with tumor proliferation and metastasis.

WGCNA

The 710 differentially expressed genes were performed WGCNA, and 532 genes were reserved based on weighted > 0.05 and minimum module size of 30 genes for further analysis. The results showed that these genes were clustered into three modules, named ME1 (155 genes), ME2 (137 genes) and ME3 (71 genes), and the other 169 genes were not classified into any module (denoted as ME0). It can be seen from Figure 4A that genes belonging to different modules were located at different branches in the hierarchical clustering tree, and the clustering results showed obvious modularity. The expression of genes belonging to the same module was highly correlated, and the hierarchical clustering heatmap presented an obvious block boarded diagonal form (Fig. 4B).

It can be seen from the topological structure of the WGCNA network that genes belonging to the same module were

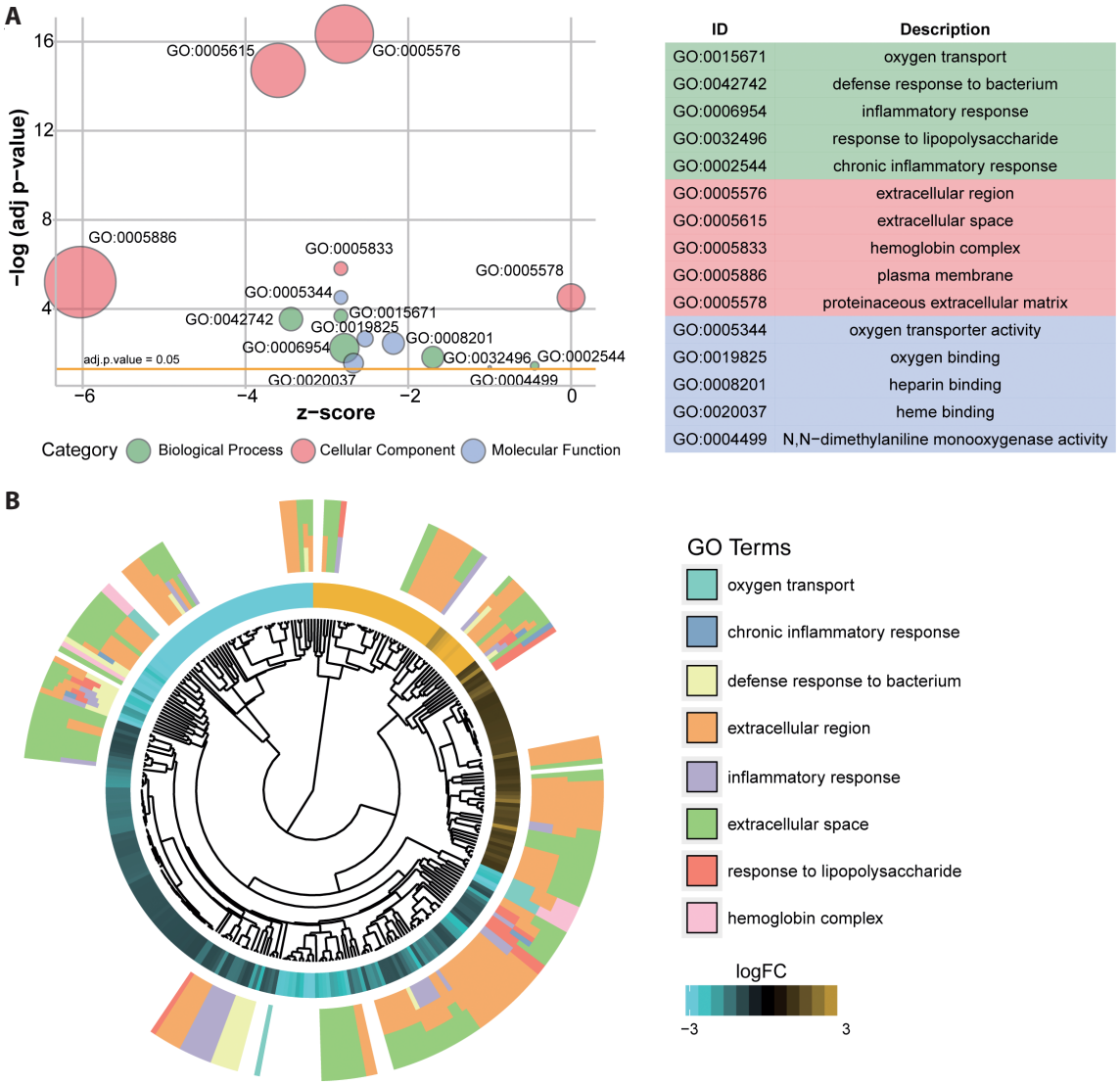

Figure 2. Results of GO enrichment analysis. **A.** The differential expression (z-score), significance (Benjamini) and the number of corresponding genes (circle size) for the top 5 biological process, cellular component and molecular function terms. **B.** For the top 8 GO terms selected, the phylogenetic tree was constructed by clustering the genes according to their occurrence in the term.

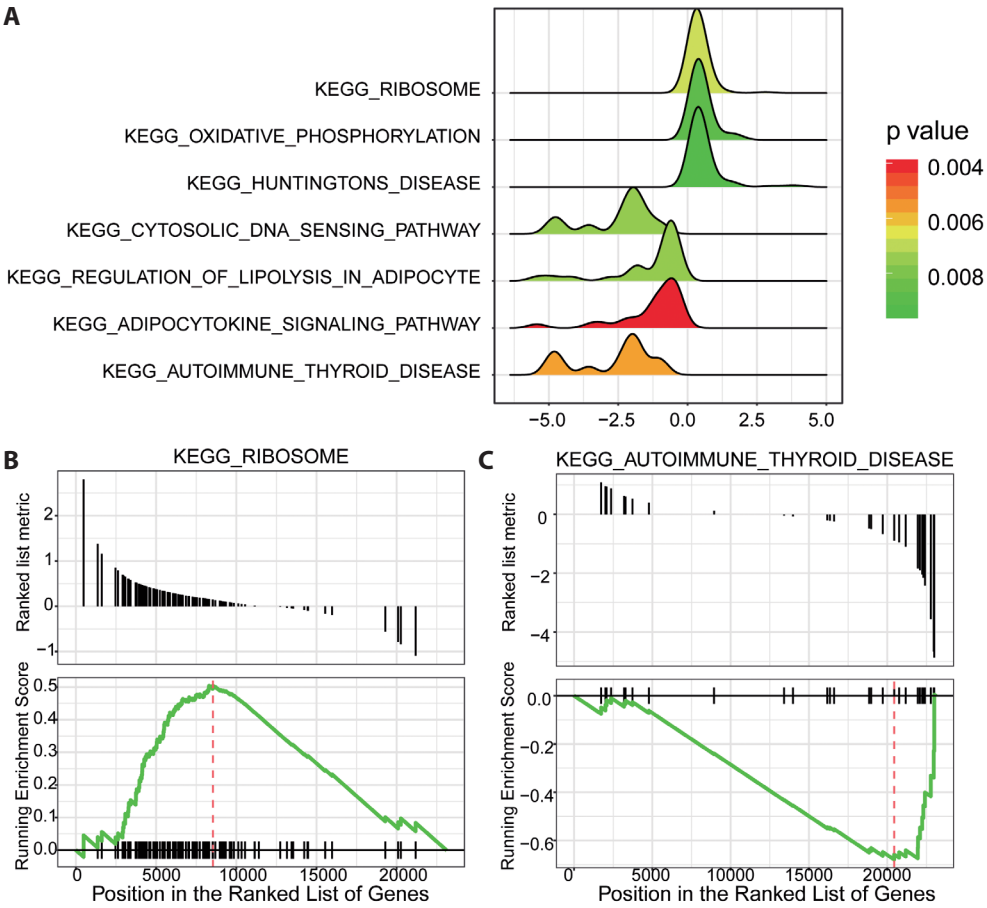

Figure 3. Enrichment results of GSEA pathway. **A.** The joyplot of the upregulated pathway. **B.** The upregulated pathway of KEGG ribosome. **C.** The downregulated pathway of KEGG autoimmune thyroid diseases.

obviously clustered into clusters, and the connectivity degree of gene within the module was much higher than that between the modules. Besides, the genes among different modules also had partial functional relationships (Fig. 4C). Additionally, there were a large number of highly connected genes (hub genes) in the ME1 module. In the ME2 module, the genes with top 10 connectivity degrees were ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (*ST3GAL4*), uridine-cytidine kinase 2 (*UCK2*), macrophage migration inhibitory factor (*MIF*), phosphoserine aminotransferase 1 (*PSAT1*), MLLT11 transcription factor 7 cofactor (*MLLT11*), homeobox D13 (*HOXD13*), mitochondrial ribosomal protein L36 (*MRPL36*), *LOC84856*, visinin like 1 (*VSNL1*) and glypican 1 (*GPC1*).

For the gene modules ME1, ME2 and ME3, the correlation between them and tumor was calculated and the false discovery rate method was used for multiple test correction. As shown in Table 1, the correlation between ME2 and tumor was as high as 0.78. The genes in ME1 and ME2 were negatively associated with tumor progression (correlation = −0.54, p = 0.00127 for ME1 and correlation = −0.40, p = 0.0166 for ME3). The genes with top 5 connectivity degrees in ME1 were glycophorin A (*GYPA*), acyl-CoA synthetase member 6 (*ASCL6*), Rh associated glycoprotein (*RHAG*), schlafen family member 14 (*SLFN14*) and myeloperoxidase (MPO). Those in ME3 were perilipin 1 (*PLIN1*), thyroid hormone responsive (*THRSP*), alcohol dehydrogenase 1B (*ADH1B*), glycerol-3-phosphate dehydrogenase 1 (*GPD1*) and *PLIN4*. The scatter plot and regression analysis results of gene-module correlation and gene-tumor correlation also indicated that this module was closely related to tumor (Fig. 4D).

lncRNA-mRNA co-expression network

For the 7 differentially expressed lncRNAs and 559 differentially expressed mRNAs, we calculated their correlation

Table 1. The correlation between modules ME1, ME2 and ME3 and tumor

Group	Correlation	adj.*p*.value (FDR)
ME1 *vs.* tumor	−0.5420891	1.27×10^{-3}
ME2 *vs.* tumor	0.7788899	6.63×10^{-8}
ME3 *vs.* tumor	−0.3966235	1.66×10^{-2}

adj.*p*.value, adjusted *p* value; FDR, false discovery rate.

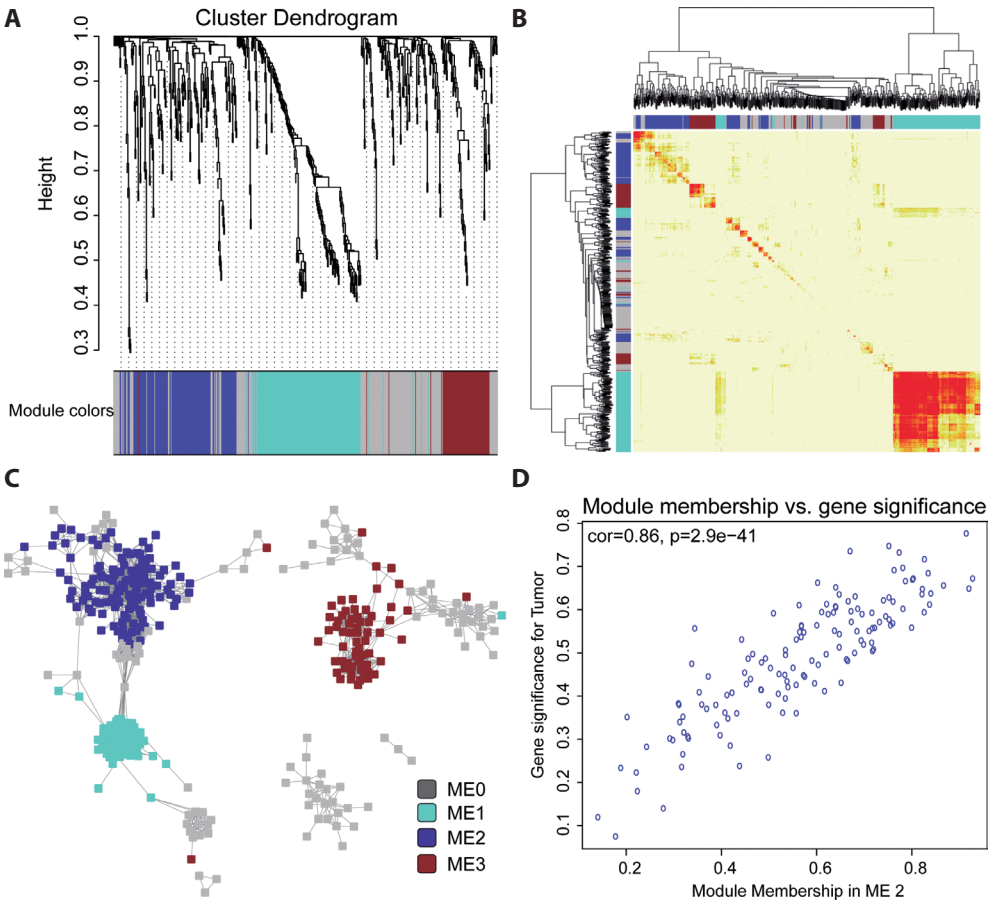

Figure 4. Results of WGCNA analysis. **A.** Gene hierarchical clustering tree. The genes were clustered and labeled according to their expression values in each sample. **B.** Hierarchical clustering heatmap. Hierarchical clustering was performed for the genes in rows and columns. The color in the heatmap indicated the correlation degree between the expression values of the two genes: the darker the color, the higher the degree of co-expression of the two genes. **C.** Gene module and co-expression network. The edge weight of 0.05 was used as the threshold value for screening, and points of different colors indicated that genes belonged to different modules. **D.** Gene-module correlation and gene-tumor correlation. X-axis represents the degree of correlation between genes and this module in the ME2 module, and Y-axis represents the degree of correlation between genes and tumor.

coefficient between each other. The gene pairs with $|r| \geq 0.6$ and adj.p.value < 0.05 were screened. The constructed co-expression network included 504 nodes (500 mRNAs and 4 lncRNAs) and 7026 edges (Fig. 5A). Gene pairs containing lncRNA were extracted from the co-expression network and obtained the subnetwork, as shown in Figure 5B. In this subnetwork, there were 12 mRNAs (phosphatidylethanolamine N-methyltransferase (*PEMT*), collagen type X alpha 1chain (*COL10A1*), glutathione S-transferase alpha 1 (*GSTA1*), etc.) that were synergistically expressed with TTTY14. GO enrichment analysis of the 12 mRNAs showed that these genes were enriched in functions related to cell proliferation (p =

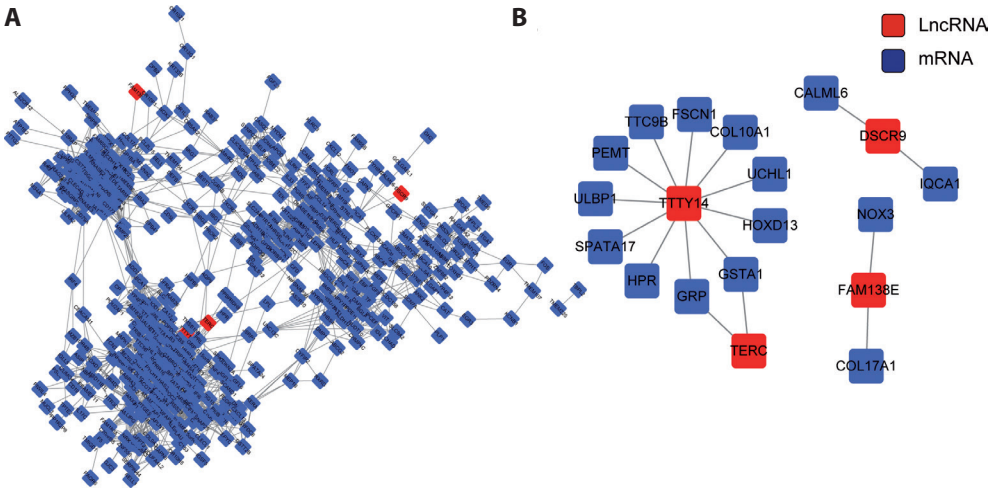

Figure 5. Gene co-expression network. **A.** lncRNA-mRNA co-expression network. **B.** Subnetworks that contains lncRNAs.

0.0034), cell projection ($p = 0.052$), sulfur metabolism ($p = 0.082$) and extracellular region ($p = 0.092$).

Discussion

In this study, 703 differentially expressed mRNAs and 7 differentially expressed lncRNAs were identified between tumor and normal tissues. The differentially expressed mRNAs were significantly enriched in functions associated with inflammatory response and ribosome pathway. WGCNA revealed that ME2 module had a high correlation with tumor, and *ST3GAL4*, *UCK2*, *PSAT1* etc. had higher connectivity degrees in this module. lncRNA-mRNA co-expression network showed that 12 mRNAs, such as *PEMT*, *COL10A1* and *GSTA1*, were synergistically expressed with lncRNA TTTY14.

GO enrichment analysis of the differentially expressed mRNAs showed that a total of 6 biological process terms, 15 cellular component terms and 8 molecular function terms were significantly enriched. The biological process terms included inflammatory response, oxygen transport, and defense response to bacterium, lipopolysaccharide, and fungus. Chronic inflammation is emerging as a hallmark of cancers, and many tumors present characteristics of chronic inflammation or arise prolonged inflammation throughout their progression (Hanahan and Weinberg 2011; Balkwill and Mantovani 2012). Inflammatory microenvironment can promote tumor development *via* altering responses to chemotherapeutic agents, promoting metastasis and angiogenesis, and subverting adaptive immune responses (Mantovani et al. 2008). During chemotherapy of cancer, addition of anti-inflammatory drugs is demonstrated to be an effective treatment to increase the survival of patients (Gotwals et al. 2017). In this study, the differentially expressed mRNAs, such as *MIF*, C-X-C motif chemokine receptor 1 (*CXCR1*), C-X-C motif chemokine ligand 12 (*CXCL12*) and TNF receptor superfamily member 4 (*TNFRSF4*) were significantly enriched in inflammatory response associated functions. This suggested the important role of inflammation and inflammation biomarkers in osteosarcoma. In terms of cellular component, the expressed proteins were mainly located on extracellular region, extracellular space, plasma membrane, and hemoglobin complex, indicating that these genes involving cell-to-cell communication and interaction were dysregulated during osteosarcoma progression. Besides, the molecular function of dysregulated mRNAs was related to activities of peroxidase and serine endopeptidase as well as binding to oxygen or heparin. The oxygen levels of cancer cells are critical for tumor cell metabolism and cancer progression. Hypoxia exists in most solid tumors due to inadequate oxygen delivery of the abnormal vasculature (Vaupel et al. 2007). Oxygen transport agents have also been developed to reverse tumor hypoxia (Graham et al. 2018). The differentially expressed mRNAs could participate in osteosarcoma progression by regulating oxygen and heparin transport.

Ribosome is an oldest molecular machine in extant life, which is responsible for the translation of information in mRNAs into functional proteins (Noller 2012). Importantly, the hyperactivation of ribosome biogenesis, which could be initiated by the loss of tumor suppressor genes or the oncogenes, play a key role in the initiation and progression of cancer (Orsolic et al. 2016). Recent study has suggested that the drugs that inhibit ribosome biogenesis may provide a feasible approach for cancer treatment (Bruno et al. 2017). In this study, GSEA showed that ribosome had the maximum NES among the upregulated pathways, and was enriched by genes such as mitochondrial ribosomal protein L12 (*MRPL12*), *MRPL36* and ribosomal protein L10 like (*RPL10L*). The upregulation of this pathway indicated that ribosome synthesis activity was significantly increased, which may accelerate protein synthesis to promote cell proliferation in osteosarcoma.

WGCNA revealed that there was a high correlation between ME2 and tumor, and *ST3GAL4*, *UCK2*, *PSAT1* etc. had higher connectivity degrees in this module. *ST3GAL4* encodes for β-galactosidase α-2,3-sialyltransferase 4, which is involved in the biosynthesis of tumor antigens sLe(x) and sulfo-sLe(x). The expression level of *ST3GAL4* is altered in various human cancers (Roa-de La Cruz et al. 2018). *UCK2* encodes the uridine-cytidine kinase, upregulation of which is common in some types of cancer tissues (van Kuilenburg and Meinsma 2016; Malami et al. 2017). Thus, *UCK2* has been regarded as a treatment target and a biomarker for cancer prognosis. PSAT1 is an enzyme catalyzing serine biosynthesis (Baek et al. 2003). It has been suggested to be associated with tumor stage in colon cancer (Friederichs et al. 2005). Depletion of *PSAT1* has been reported to suppress the proliferation of breast cancer cells (Possemato et al. 2011).

The genes with top 5 connectivity degrees in ME1 were *GYPA*, *ASCL6*, *RHAG*, *SLFN14* and MPO. Those in ME3 were *PLIN1*, *THRSP*, *ADH1B*, *GPD1* and *PLIN4*. ASCLs are necessary for phospholipid remodeling, fatty acid degradation and production of long acyl-CoA esters (Soupene and Kuipers 2008). SLFN14 belongs to the SLFN family of proteins, which were demonstrated to exert anti-melanoma effects of interferon α and involve in the regulation of cell invasion (Katsoulidis et al. 2010). MPO is an endogenous metabolic/oxidative lysosomal enzyme which plays and important role in carcinogenesis (Klebanoff 1999). Polymorphism of MPO is associated with cancer risk (Yang et al. 2017). PLIN1 and PLN4 are members of the PAT protein family that play distinct roles in regulating both triglyceride storage and lipolysis in adipocytes. PLIN1 was also reported to of prognostic significance in human breast cancer and might be a potentially new gene therapy target (Zhou et al. 2016). THRSP is a thyroid-hormone-inducible and

carbohydrate-inducible and protein, functions to activate genes involved in fatty-acid synthesis enzymes (Zhang et al. 2011). Evidence suggested it plays a role in tumorigenesis by regulating lipogenic enzymes (Kinlaw et al. 2006). Alcohol consumption is a major risk factor for cancer development. ADH1B polymorphism was significantly associated with decreased overall cancer risk (Polimanti and Gelernter 2018; Tan and Ning 2019). GPD1 was reported to enhance the anticancer effect of metformin by through synergistic inhibition of mitochondrial function (Xie et al. 2020). Taken together, we speculated that these genes may sever as key biomarkers in osteosarcoma.

Presently, the co-expression model that integrates lncRNAs and protein-coding genes are widely used to investigate the functions of lncRNAs in biological processes and cancers (Guo et al. 2012). The present study also constructed such a co-expression network. The result showed that 12 mRNAs, such as *PEMT, COL10A1* and *GSTA1*, were synergistically expressed with lncRNA TTTY14. A study reported that this TTTY14 is a prognostic biomarker of gastric cancer (Miao et al. 2017). Besides, it is closely related to the progression of various types of cancers, like lung cancer, colorectal cancer, and oropharyngeal squamous cell carcinoma (Boscolo-Rizzo et al. 2017). However, the function of this lncRNA is widely unknown in osteosarcoma. Interestingly, *PEMT*, *COL10A1* and *GSTA1* have been demonstrated to be implicated in cancer development (Li et al. 2014; Matic et al. 2016; Huang et al. 2018). Further, GO enrichment analysis showed that these genes were associated with functions of cell proliferation, cell projection, sulfur metabolism and extracellular region, which further indicated that lncRNA TTTY14 may regulate these functions in osteosarcoma tumor cells, affecting the proliferation and intercellular interactions of tumor cells by interacting with *PEMT*, *COL10A1* and *GSTA1*.

There are inevitably limitations in this study. Though the reliability of WGCNA has been validated by multiple studies, the lncRNA-mRNA interactions are still needed to be validated in *in vitro* and *in vivo* studies. Further researches are warranted to perform to investigate the interaction between lncRNA TTTY14 and its co-expressed mRNAs.

Conclusion

In conclusion, we identified 7 crucial lncRNAs and 703 mRNAs participated in osteosarcoma progression by involving in inflammatory response and ribosome synthesis. WGCNA demonstrated that lncRNA TTTY14 may affect the proliferation and intercellular interactions of tumor cells in osteosarcoma by cooperative expression with *PEMT*, *COL10A1*, *GSTA1*, etc. *ST3GAL4*, *UCK2*, *PSAT1* as well as TTTY14 may serve as key biomarkers in osteosarcoma. Further animal or clinical experiments are needed to verify our findings.

Availability of data and materials. The dataset analyzed in this study is available in GEO database with accession number of GSE99671.

Conflict of interest. The authors declare that they have no competing interests.

Author contributions. DWL and GBL designed the study, GBL and GYL analyzed and interpreted the patient data; GYL and JY performed the histological examination; JY interpreted the histological data. GBL and GYL were major contributors of manuscript writing. DWL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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