Key genes with prognostic values in suppression of osteosarcoma metastasis using comprehensive analysis

Mi Li†, Xin Jin‡, Hao Li†, Gang Wu‡, Shanshan Wang‡, Caihong Yang†* and Sisi Deng‡*

Abstract

Background: Osteosarcoma is a primary malignant tumor originating from mesenchymal tissue, with a poor distant metastasis prognosis. The molecular mechanisms of osteosarcoma metastasis are extremely complicated.

Methods: A public data series (GSE21257) was used to identify differentially expressed genes (DEGs) in osteosarcoma patients that did, or did not, develop metastases. Functional enrichment analysis, a protein-protein interaction network, and survival analysis of DEGs were performed. DEGs with a prognostic value were considered as candidate genes and their functional predictions, different expression in normal and malignant tissues, and immune infiltration were analyzed.

Results: The DEGs were mainly enriched in the immune response. Three candidate genes (ALOX5AP, CD74, and FCGR2A) were found, all of which were expressed at higher levels in lungs and lymph nodes than in matched cancer tissues and were probably expressed in the microenvironment.

Conclusions: Candidate genes can help us understand the molecular mechanisms underlying osteosarcoma metastasis and provide targets for future research.

Keywords: Osteosarcoma, Metastasis, Prognosis, Protein-protein interaction network, Differentially expressed genes

Background

Osteosarcoma is a primary malignant tumor originating from mesenchymal tissue. The annual incidence is similar worldwide, ranging from 1 to 4 in 1 million. Although the overall incidence of osteosarcoma is not high, it is the most common type of bone and soft tissue tumors, accounting for 40.51% of primary malignant bone tumors. With improvements in limb salvage surgery and neoadjuvant chemotherapy, the 5-year survival rate of non-metastatic patients is about 65–70% [1]. Unfortunately, distant metastases are found in about 20% of patients, 90% of which are lung metastases [2]. Once distant metastasis occurs, the 5-year survival rate is only 15–30% [3–5]. However, the mechanisms of osteosarcoma metastasis are still largely unknown.

In recent years, bioinformatics has been widely used to reveal tumor progression and the internal mechanism of carcinogenesis at the genome level for many cancer types. In particular, there are many bioinformatics web tools that can help us analyze relevant data, with standardized and visual results. Although microarray data for osteosarcoma are still limited, some hidden and interesting information like the expression of key genes [6–8], microRNAs [9] and co-expression modules [10] in osteosarcoma and drug resistance in osteosarcoma patients [11] could be found.

In this study, a series of mRNA data was analyzed to obtain differentially expressed genes (DEGs) between osteosarcoma patients that did, or did not, develop metastases. Subsequently, a protein-protein interaction (PPI) network of the DEGs was constructed. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, and survival analysis were used to identify candidate genes.
Furthermore, we analyzed function predictions, different expression in normal and malignant human tissues, and immune infiltration analysis of the candidate genes to confirm their function and distribution. In conclusion, 24 DEGs and three candidate genes were identified.

Methods
Identification of DEGs and PPI network construction
A public series submitted by Buddingh et al. in 2011, GSE21257 [12], was downloaded from the Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo, RRID: SCR_005012) [13]. The series contains 53 pre-chemotherapy biopsy samples from osteosarcoma patients that developed metastases (n = 34) and that did not develop metastases within 5 yrs. (n = 19). The biopsy tissue contained the tumor cells and microenvironment around the tumor. All the expression data were analyzed via the R language (version 3.5.1) BIOCONDUCTOR package, and the DEGs were screened using the LIMMA package at a statistical significance Benjamini and Hochberg false discovery rate-adjusted p-value cutoff of 0.05 and an absolute value of fold change greater than 2. The online Search Tool for the Retrieval of Interacting Genes (STRING, http://string-db.org, RRID: SCR_005223) [14] is a database of known and predicted protein-protein interactions. We used STRING to find observed co-expression of the DEGs in humans and constructed a PPI network of the DEGs with statistical significance of interaction scores > 0.4 (medium confidence score).

GO and pathway enrichment
The GO and KEGG pathway enrichment analyses were performed using DAVID (https://david.ncifcrf.gov/, RRID: SCR_001881) [15]. The biological process (BP) analysis, cellular component (CC) analysis, molecular function (MF) analysis [16], and KEGG [17] pathway enrichment analysis of the DEGs were carried out and p-values < 0.05 were considered to indicate statistical significance. Moreover, a biological process analysis of the hub genes was constructed and visualized using the Biological Networks Gene Ontology tool (BiNGO, RRID: SCR_005736) [18] plugin of Cytoscape (version 3.6.1, RRID: SCR_003032) [19].

Survival analysis of the DEGs
PROGgeneV2 (http://genomics.jefferson.edu/proggene) [20] is a tool that can be used with publicly available data to study the prognostic implications of genes. All the DEGs were input into the database separately and overall survival plots (Kaplan Meier, KM plots) were created based on the cohort divided at the median of the given gene expression. PROGgeneV2 uses the SURVIVAL package of R for the hypothesis test. The DEGs that had p-values < 0.05 were considered as candidate genes and were analyzed further.

Function predictions of the candidate genes
GeneMANIA (http://www.genemania.org, RRID: SCR_005709) [21] is a flexible, user-friendly open-source tool. Besides constructing the PPI network, the web tool can display an interactive functional association network, illustrating the relationships among genes. The advanced statistical options used were max resultant genes = 20, max resultant attributes = 10, and the automatically selected network weighting method. These analyses were conducted using the Homo sapiens database.

Different expression of candidate genes in normal and malignant human tissues
The SAGE Anatomic Viewer, part of the online Serial Analysis of Gene Expression database (SAGE, http://www.ncbi.nlm.nih.gov/SAGE, RRID: SCR_000796) [22], was used to display candidate gene expression in normal and malignant human tissues. The related expression levels were based on the analysis of counts of SAGE tags, ordered by color.

Immune infiltration analysis of the candidate genes
Tumor IMmune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) [23] is a comprehensive web server for systematic analysis of immune infiltrates across diverse cancer types. When we input the candidate gene symbols for at least one cancer type, scatter-plots were generated and displayed showing the purity-corrected partial Spearman’s correlations and statistical significance. Tumor purity is expected to have negative associations with high levels of expression in the microenvironment, while the opposite is true for the tumor cells. Unfortunately, there is no available data for osteosarcoma, so we chose SARC (sarcoma), OV (ovarian serous cystadenocarcinoma), LUSC (lung squamous cell carcinoma), LIHC (liver hepatocellular carcinoma), and BRCA (breast invasive carcinoma) as the multi-cancer types.

Results
Identification of DEGs and PPI network construction
Only 24 downregulated DEGs were recognized in the osteosarcoma patients that developed metastases, and no upregulated genes were found in the profiles (Fig. 1a), meaning that the DEGs protect patients from metastases. Detailed information for the DEGs is shown in Table 1. The co-expressed DEGs in humans are shown in Fig. 1b. The PPI network of the DEGs is shown in Fig. 1c.
GO and pathway enrichment
The results of the biological classification of the DEGs, and functional and pathway enrichment analyses are shown in Fig. 2 (details are shown in Tables 2 and 3). The results of the biological process analysis of the DEGs is shown in Fig. 1d. GO analysis showed that in the BP ontology (Fig. 2a), immune response (10 genes) and T cell co-stimulation (6 genes) constituted the most significantly enriched terms. In the CC ontology (Fig. 2b), the most significantly enriched terms were involved in MHC class II protein complex (9 genes) and the lysosomal membrane (9 genes). In the MF ontology (Fig. 2c), the most...
significantly enriched terms were involved in MHC class II receptor activity (7 genes), MHC class II protein complex binding (5 genes), and peptide antigen binding (5 genes). In the KEGG pathways (Fig. 2d), the most significantly enriched terms were shown as tuberculosis (11 genes) and systemic lupus erythematosus (11 genes).

Survival analysis of the DEGs
Among the 24 DEGs, overall survival plots were obtained for 15 genes, as shown in Fig. 3. The high expression group of 15 DEGs would have better survival than the low expression group. However, only three of these were significant (< 0.05), namely ALOX5AP, CD74, and FCGR2A. These were selected as the candidate genes for further analyses. The gene expression of the candidate genes could be found in the Additional file 1: Table S1.

Function predictions for the candidate genes
An interactive functional association network constructed by GeneMANIA revealed correlations among genes for the candidate genes. The gene set enriched for ALOX5AP is responsible mainly for eicosanoid and fatty acid derivative biosynthetic processes (Fig. 4a). Meanwhile, the gene set enriched for CD74 is responsible mainly for positive regulation of lymphocyte activation and leukocyte activation (Fig. 4b), and the gene set enriched for FCGR2A is responsible for immune response-regulating cell surface receptor signaling pathways, and Fc receptor signaling pathways (Fig. 4c). Moreover, the gene set enriched for the three genes is responsible mainly for antigen processing and presentation of exogenous peptide antigens via MHC class II, antigen processing, and presentation of peptide antigens via MHC class II (Fig. 4d). Compared to the functional analyses of the DEGs, the enriched functions of the candidate genes also have their own characteristics.

Different expression of candidate genes in normal and malignant human tissues
The expression profiles of the three candidate genes in human tissue were displayed using SAGE. As
shown, ALOX5AP mRNA in lung, liver, breast, peritoneum, and lymph node tissues displayed higher levels than in the matched cancer tissues (Fig. 5a). CD74 mRNA in brain, retina, lung, and lymph nodes displayed higher levels than in the matched cancer tissues (Fig. 5b), while FCGR2A mRNA in thyroid, lung, kidney, peritoneum, and lymph node tissues displayed higher levels than in the matched cancer tissues (Fig. 5c). All the candidate genes were expressed at higher levels in lung and lymph node tissues than in the matched cancer tissues.

Immune infiltration analysis of the candidate genes

In the five cancer types we selected, the expression levels of the three candidate genes were all negatively associated with tumor purity (Fig. 6). It can be inferred from this result that all three candidate genes are probably expressed in the microenvironment, not in the tumor cells.

The data that support the findings of this study were generated at GSE21257 [12] in GEO. Derived data supporting the findings of this study are available from the corresponding author on request.

Discussion

Osteosarcoma metastasis is a complex process of interaction between multiple genes and multiple signaling pathways in tumor cells and stromal cells. The deletion of the p53 gene and activation of the Notch pathway in osteosarcoma cells may contribute to invasion and metastasis [24]. Induction of Src-family tyrosine kinase (SFK) and the synergy of metal matrix protease-2, 9 (MMPs-2, 9), may help osteosarcoma cells degrade the extracellular matrix and enter the blood circulation by activating the Wnt/beta-catenin signaling pathway [25]. Meanwhile, SFK activates PI3K/AKT and Ras/MAPK...
| Category           | GO ID     | GO Term                                                                 | Count | FDR    | Log P    | Genes                                                                 |
|--------------------|-----------|-------------------------------------------------------------------------|-------|--------|----------|-----------------------------------------------------------------------|
| Biological Process | 0002504   | antigen processing and presentation of peptide or polysaccharide antigen via MHC class II | 8     | 1.62E-14 | −16.8846 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRB4 |
| Biological Process | 0019886   | antigen processing and presentation of exogenous peptide antigen via MHC class II | 9     | 5.29E-11 | −13.369  | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DQA1, HLA-DRB4 |
| Biological Process | 0019882   | antigen processing and presentation                                       | 7     | 3.03E-08 | −10.6113 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, CD74, HLA-DQA1, HLA-DRB4 |
| Biological Process | 0006955   | immune response                                                           | 10    | 3.29E-07 | −9.57522 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DQA1, HLA-DRB4 |
| Biological Process | 0002503   | peptide antigen assembly with MHC class II protein complex                | 4     | 1.52E-05 | −7.91138 | HLA-DRB4, HLA-DMB, HLA-DMA, HLA-DRB4 |
| Cellular Component | 0042613   | MHC class II protein complex                                               | 9     | 2.13E-16 | −18.6699 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DQA1, HLA-DRB4 |
| Cellular Component | 0071556   | integral component of luminal side of endoplasmic reticulum membrane      | 6     | 1.41E-07 | −9.4824  | HLA-DQB1, HLA-DRB4, HLA-DPA1, CD74, HLA-DQA1, HLA-DRB4 |
| Cellular Component | 0005765   | lysosomal membrane                                                         | 9     | 4.03E-07 | −9.39244 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DQA1, HLA-DRB4 |
| Cellular Component | 0030658   | transport vesicle membrane                                                 | 6     | 5.93E-07 | −9.2251  | HLA-DQB1, HLA-DRB4, HLA-DPA1, CD74, HLA-DQA1, HLA-DRB4 |
| Cellular Component | 0030669   | clathrin-coated endocytic vesicle membrane                                | 6     | 8.83E-07 | −9.05199 | HLA-DQB1, HLA-DRB4, HLA-DPA1, CD74, HLA-DQA1, HLA-DRB4 |
| Molecular Function | 0032395   | MHC class II receptor activity                                             | 7     | 5.72E-12 | −14.2221 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRB4 |
| Molecular Function | 0023026   | MHC class II protein complex binding                                       | 5     | 2.47E-06 | −8.58782 | HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DRB4 |
| Molecular Function | 0042605   | peptide antigen binding                                                   | 5     | 2.75E-05 | −7.54062 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DQA1, HLA-DRB4 |
| Molecular Function | 0004051   | arachidonate 5-lipoxygenase activity                                       | 2     | 2.237376 | −2.62558 | ALOX5AP, ALOX5 |
| Pathway ID   | Pathway Name                                      | Gene Count | FDR    | LogP   | Genes                                                                 |
|-------------|---------------------------------------------------|------------|--------|--------|-----------------------------------------------------------------------|
| hsa05150    | Staphylococcus aureus infection                   | 10         | 6.33E-13 | -15.21 | HLA-DQB1, C1QA, HLA-DRB4, HLA-DPA1, FCGR2A, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05332    | Graft-versus-host disease                         | 9          | 1.37E-12 | -14.85 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05330    | Allograft rejection                               | 9          | 3.67E-12 | -14.41 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa04940    | Type I diabetes mellitus                          | 9          | 1.11E-11 | -13.93 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa04672    | Intestinal immune network for IgA production     | 9          | 2.96E-11 | -13.50 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05322    | Systemic lupus erythematosus                      | 11         | 3.85E-11 | -13.39 | HLA-DQB1, C1QA, CD86, HLA-DRB4, HLA-DPA1, FCGR2A, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05320    | Autoimmune thyroid disease                        | 9          | 7.06E-11 | -13.13 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05310    | Asthma                                            | 8          | 1.49E-10 | -12.80 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05416    | Viral myocarditis                                 | 9          | 1.54E-10 | -12.79 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05152    | Tuberculosis                                      | 11         | 6.54E-10 | -12.16 | HLA-DQB1, HLA-DRB4, HLA-DPA1, FCGR2A, HLA-DMB, HLA-DOA, HLA-DMA, CD14, CD74, HLA-DQA1, HLA-DRA |
| hsa05140    | Leishmaniasis                                     | 9          | 9.79E-10 | -11.98 | HLA-DQB1, HLA-DRB4, HLA-DPA1, FCGR2A, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa04612    | Antigen processing and presentation               | 9          | 1.73E-09 | -11.74 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DQA1, HLA-DRA |
| hsa05323    | Rheumatoid arthritis                              | 9          | 5.81E-09 | -11.21 | HLA-DQB1, CD86, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa04145    | Phagosome                                         | 10         | 8.33E-09 | -11.06 | HLA-DQB1, HLA-DRB4, HLA-DPA1, FCGR2A, HLA-DMB, HLA-DOA, HLA-DMA, CD14, HLA-DQA1, HLA-DRA |
| hsa05145    | Toxoplasmosis                                     | 9          | 3.62E-08 | -10.42 | HLA-DQB1, HLA-DRB4, HLA-DPA1, ALOX5, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05321    | Inflammatory bowel disease (IBD)                  | 9          | 4.36E-08 | -10.34 | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa04514    | Cell adhesion molecules (CAMs)                    | 8          | 2.86E-07 | -9.52  | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05168    | Herpes simplex infection                          | 9          | 2.18E-06 | -8.64  | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, CD74, HLA-DQA1, HLA-DRA |
| hsa05164    | Influenza A                                       | 8          | 5.24E-05 | -7.26  | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05166    | HTLV-I infection                                  | 8          | 7.01E-04 | -6.13  | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DMB, HLA-DOA, HLA-DMA, HLA-DQA1, HLA-DRA |
| hsa05169    | Epstein-Barr virus infection                      | 5          | 0.13728 | -3.84  | HLA-DQB1, HLA-DRB4, HLA-DPA1, HLA-DQA1, HLA-DRA |
| hsa04640    | Hematopoietic cell lineage                        | 3          | 14.8897 | -1.77  | HLA-DRB4, CD14, HLA-DRA |
signaling pathways to avoid apoptosis in osteosarcoma cells [26].

Buddingh et al. first reported the series in 2011 [12] which we analyzed in this study. They also compared patients that did, or did not, develop metastases within 5 years and identified 14 upregulated and 118 downregulated genes in patients that developed metastases, with an only statistical criterion of adjust \( p < 0.05 \). Buddingh proved that these genes were expressed by tumor stroma and not by tumor cells by experiment. Almost half of these genes were attributed to macrophage function. Furthermore, the authors proposed that tumor-associated macrophages (TAM) in the tumor-microenvironment have an antimetastatic effect, which can improve survival in osteosarcoma. This is a notable work. However, the considered statistical criteria was just only the \( p \)-value and may produce some false-positive results. Meanwhile, the authors focused on the antimetastatic function of TAM and provided a detailed argument to support this. They did not identify the key molecules played a role in this process which would be benefit for future researchers. In our study, only 24 downregulated DEGs were recognized with a statistical significanc of adjust \( p < 0.05 \) and absolute value of fold change > 2. These DEGs have greater statistical significance than those in the previous study. Besides that, we proposed three prognostic candidate genes would play an important role in the patients who did develop metastases within 5 years.

GO and KEGG pathway enrichment analyses revealed that changes in the DEGs mainly occurred in the MHC class II protein complex, immune response, and antigen processing and presentation. In other words, immune infiltrates or immune responses in the local microenvironment play an important role in osteosarcoma metastasis. Previous studies have reported that during metastasis, tumor-infiltrating lymphocytes (TILs) can be detected at a higher level than in normal tissue [27], and patients with higher T-lymphocyte infiltration showed improved survival [28, 29]. It was proposed that some portion of the T-cells (like TILs) would act against tumor cells with a higher specific immunological reactivity than the non-infiltrating lymphocytes [27]. Moreover, programmed cell death protein 1 (PD-1) showed increased expressed in TIL [30] and peripheral CD4\(^+\) and CD8\(^+\) T-lymphocytes [31]. Based on this result, the inhibition of the PD-1/PDL-1 interaction would lead to a decreased tumor burden in osteosarcoma-bearing mice [32]. Overall, these theories are in agreement with our results.

Three candidate genes with prognostic value—namely ALOX5AP, CD74, and FCGR2A—were discovered. Interestingly, all the candidate genes showed higher expression in lung and lymph node tissues than in the matched cancer tissues and were probably expressed in the microenvironment, not in the tumor cells. This result is consistent with that of previous studies; the candidate genes are reportedly linked to tumor cells. The change in ALOX5AP expression can cause oxidative stress, which has some effects on human leukemia [33]. Codreanu et al. reported that...
Fig. 4 Protein-protein interaction network of ALOX5AP (a), CD74 (b), and FCGR2A (c) candidate genes (d). Different colors of the network edges indicate the bioinformatics method applied; the different colors for the network nodes indicate the biological functions of the set of enrichment genes.
ALOX5AP could be a noninvasive candidate biomarker for lung cancer with global and targeted proteomics [34]. Knights et al. identified ALOX5AP as associated with the pharmacokinetics of gemcitabine, which is an approved anti-cancer drug [35]. Meanwhile, high expression of CD74 would cause functional HLA class II processing in brain metastatic tumor cells, with a better prognosis [36]. Figueiredo et al. reported that MIF-CD74 signaling regulates the antitumor immune response of macrophages and dendritic cells in metastatic melanoma [37]. Ekmekcioglu et al. found that CD74 is associated with overall survival and recurrence-free survival in stage III melanoma, and could be a useful prognostic tumor marker [38]. Furthermore, FCGR2A is reportedly associated with the pharmacodynamics of monoclonal antibodies in different cancer types, such as colorectal cancer [39], breast cancer [40], and metastatic squamous cell head and neck cancer [41]. However, a search of the published literature revealed that there are few studies about the candidate genes for osteosarcoma. This observation suggests that the candidate genes may require further research to reveal the mechanisms of osteosarcoma metastasis.

This bioinformatics study provides information on the DEGs and candidate genes that protect osteosarcoma patients from metastasis, which could inform future research. However, we must recognize that the
roles of the candidate genes are still unknown. Additional well-designed experiments and analyses are required to reveal these mechanisms. In addition, all the results from this study were obtained in silicon; in vivo and in vitro experiments are necessary to test the functions of these DEGs. A note that if we could include some critical details of the surrounding muscle tissue, we might better analyze the mechanism of osteosarcoma metastasis.

**Conclusions**

In conclusion, we identified 24 DEGs, of which three candidate genes may be involved in the processes that protect osteosarcoma patients from metastasis. The molecules we found are potential targets for future research on osteosarcoma immunity. Furthermore, our results contribute to the identified biomarkers for osteosarcoma metastasis.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s12885-020-6542-z.

**Abbreviations**

DEGs: Differentially expressed genes; GEO: Gene Expression Omnibus; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: Protein-protein interaction; SAGE: Serial Analysis of Gene Expression; STRING: Search Tool for the Retrieval of Interacting Genes; TIMER: Tumor IMmune Estimation Resource

**Acknowledgements**

We thank Lao Xinyuan, the CEO of helixlife, for his guidance and help in our scientific research work.

**Authors’ contributions**

LM and JX analyzed the data, WS download the data from GEO database. LH provided the help of the R language. YC and WG suggested the online tools. DS and YC designed the project. DS selected the analyzed results and wrote the paper. All authors read and approved the final manuscript.

**Funding**

Not applicable.

**Availability of data and materials**

The data that support the findings of this study were generated at GSE21257(Buddingh et al., 2011) in GEO. Derived data supporting the findings of this study are available from the corresponding author on reasonable request.
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Orthopedics, Tongji Hospital, Tongji Medical College, HuaZhong University of Science and Technology, Wuhan 430030, China. 2Department of Digestive Surgical Oncology, Union Hospital, Tongji Medical College, HuaZhong University of Science and Technology, Wuhan 430022, China. 3Cancer Center, Union Hospital, Tongji Medical College, HuaZhong University of Science and Technology, Wuhan 430022, China.

Received: 10 July 2019 Accepted: 14 January 2020
Published online: 28 January 2020

References
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7–30.
2. Luetke A, Meyers PA, Lewis I, Juergens H. Osteosarcoma treatment - where do we stand? A state of the art review. Cancer Treat Rev. 2014;40(4):523–32.
3. Gianfrancone DM, Mirabello L, Savage SA. Germline and somatic genetics of osteosarcoma – connecting anatomy, biology and therapy. Nat Rev Endocrinol. 2017;13(8):480–91.
4. Lin YH, Jewell BE, Gingold J, Lu L, Zhao R, Wang LL, Lee DF. Osteosarcoma: molecular pathogenesis and iPSC modeling. Trends Mol Med. 2017;23(8):737–55.
5. Whelan JS, Davis LE. Osteosarcoma, Chondrosarcoma, and Chordoma. J Clin Oncol. 2018;36(2):188–92.
6. Dong B, Wang G, Yao J, Yuan P, Kang W, Zhi L, He X. Predicting novel genes and pathways associated with osteosarcoma by using bioinformatics analysis. Gene. 2017;628:32–53.
7. Li M, Jin X, Guo F, Wu G, Wu L, Deng S. Integrative analyses of key genes and regulatory elements in fluoride-affected osteosarcoma. J Cell Biochem. 2019;120(9):15397–409.
8. Liu X, Hu AX, Zhao JI, Chen FL. Identification of key gene modules in human osteosarcoma by co-expression analysis weighted gene co-expression network analysis (WGCNA). J Cell Biochem. 2017;118(11):3953–9.
9. Ma G, Zhang C, Luo W, Zhao J, Wang X, Qian Y. Construction of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44–57.
10. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO archive for functional genomics data sets–update. Nucleic Acids Res. 2013;41(Database issue):D991–5.
11. Bhuwaneshvar K, Hmiss S, Gusev Y, Madhavan S, Iyer R, Vilboux T, Deeken J, Yang E, Shankar S. Genome sequencing analysis of blood cells identifies novel pathotypes strongly associated with drug resistance in osteosarcoma patients. BMC Cancer. 2019;19(1):357.
12. Buddingh EP, Kuijler ML, Duim RA, Burger H, Ageletopoulos K, Myklebost O, Serra M, Mertens F, Hogendoorn PC, Lankester AC, et al. Tumor-infiltrating macrophages are associated with metastasis suppression in high-grade osteosarcoma: a rationale for treatment with macrophage activating agents. Clin Cancer Res. 2011;17(8):2110–9.
13. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashenko M, Marshall KA, Philippi KH, Sherman PM, Holko M, et al. NCBI GEO archive for functional genomics data sets–update. Nucleic Acids Res. 2013;41(Database issue):D991–5.
14. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45(D1):D636–D636.
15. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44–57.
16. Consortium TGO. The gene ontology project in 2008. Nucleic Acids Res. 2008;36(Database issue):D440–4.
17. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30.
18. Maere S, Heymans K, Kuiper M. BINGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics (Oxford, England). 2005;21(16):3448–56.
19. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics (Oxford, England). 2011;27(3):431–2.
20. Goswami CP, Nakshatri H. PROGeneV2 enhancements on the existing database. BMC Cancer. 2014;14:4970.
21. Sarvari R, Zarei M, Panahi P, Khorasanizadeh S. A web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77(21):e108–10.
22. Boon K, Osorio EC, Greenhut SF, Schaefer CF, Shoemaker J, Polyak K, Morin PJ, Buettow KH, Staebist R, De Souza SJ, et al. An anatomy of normal and malignant gene expression. Proc Natl Acad Sci U S A. 2002;99(17):1287–92.
23. Li T, Fan J, Wang S, Traugh N, Chen Q, Liu J, Li B, Liu XS. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77(21):e108–10.
24. Zanotti S, Canalis E. Notch signaling and the skeleton. Endocr Rev. 2016;37(3):223–53.
25. Angulo P, Kaukish G, Subramaniam D, Dandawate P, Neville K, Chastain K, Anant S. Natural compounds targeting major cell signaling pathways: a novel paradigm for osteosarcoma therapy. J Hematol Oncol. 2017;10(1):10.
26. Moriarity BS, Otto GM, Rahmann EP, Rathe SK, Wolf NK, Weg MT, Manlove LA, LaRue RS, Temiz NA. A sleeping beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and metastasis. Nat Genet. 2015;47(6):615–24.
27. Muthana M, Rodrigues S, Chen YY, Welford A, Hughes R, Tazzymann S, Essand M, Morrow F, Lewis CE. Macrophage delivery of an oncolytic virus abolishes tumor regression and metastasis after chemotherapy or irradiation. Cancer Res. 2013;73(2):490–5.
28. Fritzsche B, Fellenberg J, Moskovszky L, Sapi Z, Krenatsch T, Machado I, Poelsch J, Lehnert B, Sendroiu M, Bosch AL, et al. CD8+FOXP3+ ratio in osteosarcoma microenvironment separates survivors from non-survivors: a multicenter validated retrospective study. Oncoimmunology. 2015;4(3):e906080.
29. Koirala P, Roth ME, Gill J, Piperedi S, Chinai JM, Geller DS, Hoang BH, Park A, Fremed MA, Zang X, et al. Immune infiltration and PD-L1 expression in the tumor microenvironment are prognostic in osteosarcoma. Sci Rep. 2016;6:30093.
30. Maekawa N, Konnai S, Okawara T, Nishimori A, Ikekuchi R, Izumi Y, Takagi S, Kagawa Y, Nakajima C, Suzuki Y, et al. Immunohistochemical analysis of PD-L1 expression in canine malignant cancers and PD-L1 expression on lymphocytes in canine Oral melanoma. PLoS One. 2016;11(6):e0157176.
31. Zheng W, Xiao H, Liu H, Zhou Y. Expression of programmed death 1 is correlated with progression of osteosarcoma. APMS. 2015;123(2):102–7.
32. Lussier DM, O'Neill L, Nieves LM, McAfee MS, Holeck A, Collins AW, Dickman P, Jacobsen J, Hingorani P, Blattman JN. Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions. J Immunother (Hagerstown, Md : 1997). 2015;38(3):106.
33. Szerebi GJ, Balog JA, Demjen A, Alfoldi R, Vegi VL, Feher LZ, Man I, Kotogony E, Guban B, Batar P, et al. Imidazol[1,2-b]pyrazole-7-carboxamides Induce Apoptosis in Human Leukemia Cells at Nanomolar Concentrations. Molecules (Basel, Switzerland). 2018;23(11):2845.
34. Codreanu SG, Holcsker MD, Stebos RIC, Zimmerman LJ, Rahmann SMJ, Li M, Chen SC, Chen H, Eisenberg R, Liebler DC, et al. Identification of proteomic features to distinguish benign pulmonary nodules from lung adenocarcinoma. J Proteome Res. 2017;16(9):5266–76.
35. Knights J, Sato Y, Kaniwa N, Saito Y, Ueno H, Ramanathan M. Genetic factors associated with gemcitabine pharmacokinetics, disposition, and toxicity. Pharmacogenet Genom. 2014;24(1):15–23.
36. Zeiner PS, Zinke J, Kowalewski DJ, Bernatz S, Tichy J, Ronellenfitsch MW, Thomen F, Berger A, Forster MT, Muller A, et al. CD74 regulates complexity of tumor cell HLA class II peptide in brain metastasis and is a positive prognostic marker for patient survival. Acta Neuropathol Commun. 2018;6(1):18.
37. Figueiredo CR, Azevedo RA, Mousdell S, Resende-Lara PT, Ireland L, Santos A, Girola N, Cunha R, Schmid MC, Polonelli L, et al. Blockade of MIF-CD74 Signalling on macrophages and dendritic cells restores the
antitumour immune response against metastatic melanoma. Front Immunol. 2018;9:1132.

38. Ekmekcioglu S, Davies MA, Tanese K, Roszik J, Shin-Sim M, Bassett RL Jr, Milton DR, Woodman SE, Prieto VG, Gershewald JE, et al. Inflammatory marker testing identifies CD74 expression in melanoma tumor cells, and its expression associates with favorable survival for stage III melanoma. Clin cancer Res. 2016;22(12):3016–24.

39. Kjersem JB, Skovlund E, Ikdahl T, Guren T, Kersten C, Dalsgaard AM, Yilmaz MK, Fokstuen T, Tveit KM, Kure EH. FCGR2A and FCGR3A polymorphisms and clinical outcome in metastatic colorectal cancer patients treated with first-line 5-fluorouracil/folinic acid and oxaliplatin +/- cetuximab. BMC Cancer. 2014;14:340.

40. Gavin PG, Song N, Kim SR, Lipchik C, Johnson NL, Bandos H, Finnigan M, Rastogi P, Fehrenbacher L, Mamounas EP, et al. Association of Polymorphisms in FCGR2A and FCGR3A with degree of Trastuzumab benefit in the adjuvant treatment of ERBB2/HER2-positive breast cancer: analysis of the NSABP B-31 trial. JAMA Oncol. 2017;3(3):335–41.

41. Magnes T, Melchardt T, Hufnagl C, Weiss L, Mittemair C, Neureiter D, Kleser E, Rinnerthaler G, Roesch S, Gaggl A, et al. The influence of FCGR2A and FCGR3A polymorphisms on the survival of patients with recurrent or metastatic squamous cell head and neck cancer treated with cetuximab. Pharmacogenomics J. 2018;18(3):474–9.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.