Technology in Cancer Research & Treatment
Volume 19: 1-9

DOI: 10.1177/1533033820973278
journals.sagepub.com/home/tct
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CENPE, PRC1, TTK, and PLK4 May Play Crucial Roles in the Osteosarcoma Progression

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Abstract

Osteosarcoma (OS) is a cancerous tumor in a bone. We aimed to identify the critical genes involved in OS progression, and then try to elucidate the molecular mechanisms of this disease. The microarray data of GSE32395 was used for the present study. We analyzed differentially expressed genes (DEGs) in OS cells compared with control group by Student's t-test. The significant enriched gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathways were analyzed for upregulated genes and downregulated genes, respectively. In addition, a protein-protein interaction (PPI) network was constructed. GO and KEGG enrichment analyses were conducted for genes in the PPI network. In total, 183 DEGs, including 100 upregulated DEGs and 83 downregulated DEGs were screened. The upregulated DEGs were significantly enriched in 2 KEGG pathways, such as "Glycosaminoglycan biosynthesis-chondroitin sulfate" and the downregulated DEGs were significantly enriched in 12 pathways, including "cell adhesion molecules," "pentose phosphate pathway" and "allograft rejection." GO enrichment analysis indicated that the upregulated DEGs were significantly involved in biological process, such as "multicellular organismal metabolic process" and "limb morphogenesis," while the downregulated DEGs were significantly enriched in biological process, such as "Positive regulation of pathway-restricted SMAD protein phosphorylation." The PPI network included 84 interactions and 51 nodes. The "glycosaminoglycan biosynthesis-chondroitin sulfate pathway," "microtubule motor activityfunction," and "regulation of mitosis process" were significantly enriched by genes in PPI network. In particular, CENPE, PRC1, TTK, and PLK4 had higher degrees in the PPI network. The interactions between TTK and PLK4 as well as CENPE and PRC1 may involve in the OS development. These 4 genes might be possible biomarkers for the treatment and diagnosis of OS.

Keywords

osteosarcoma, molecular mechanisms, differentially expressed genes

Received: July 01, 2019; Revised: October 12, 2020; Accepted: October 21, 2020.

Introduction

Osteosarcoma (OS), originating from osteoblasts, is the most common bone malignant tumor, which mainly afflicts young adults or children.[1] Currently, the main therapy for OS is conventional chemotherapy, but metastatic OS exhibits resistance to conventional chemotherapy.[2] Early surgical resection combined with multi-agent chemotherapy could lead to a long-term survivor, while patients with metastatic OS had a poor prognosis with survival rate of less than 20%.[3] Consequently, researches on the molecular mechanisms of OS may helpful for the development of novel target therapies for OS patients.

Considerable researches have been conducted to investigate the molecular mechanism of OS and several genes that play important roles have been identified. For example, glycogen synthase kinase-3β (*GSK-3β*) was found to play oncogenic effect on OS cells, and that GSK-3β repression can restrain the pathway of nuclear factor-κB (NF-κB), which can lead to the

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apoptosis of OS cells.[4] Pi et al. found that Aurora-B might promote the progression of OS activating the PTK2/PI3K/Akt-NF-κB pathway.[5] Besides, cyclin-dependent kinases (CDKs) are important for cell division and cell cycle regulation, and play important roles in OS development through affecting numerous pathways, such as those of cell cycle control.[6] Moreover, it has been shown that CDK4 and CDK9 were found have potential to be prognostic marker and therapeutic target in OS.[7,8] Overall, the pathogenesis of OS is multifactorial and thus more attentions upon it are needed.

In this current study, in order to have a better understanding of OS, differentially expressed genes (DEGs) in OS cells were identified. Meanwhile, we carried out of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses for the DEGs. In addition, we built a protein-protein interaction (PPI) network to investigate the pivotal DEGs related with the progression of OS in-depth. Finally, the key genes in PPI network were validated in 4 OS cell lines. These key DEGs could be beneficial for uncovering the molecular mechanisms of OS and might be potential targets for the therapy of OS.

Materials and Methods

Sources of Data

The gene expression profile of the GSE32395 dataset including 7 OS cell lines (HOS osteosarcoma cell line CRL-1543, HOS-58 osteosarcoma cell line, U2-OS osteosarcoma cell line HTB-96, Saos osteosarcoma cell line HTB-85, MNNG/HOS osteosarcoma cell line CRL-1547, SJSA osteosarcoma cell line CRL-2098, and MG-63 osteosarcoma cell line CRL-1427, OS group) and 2 control cells (L87/4 human stem cells and hFOB 1.19 human osteoblasts, control group) was downloaded from Gene Expression Omnibus (GEO) database, the platform of which was GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version].

Data Preprocessing and Analysis of DEGs

The gene expression matrix was obtained by the preprocessing of the raw data as performed in Bioconductor AFFY package.[9] The data were preprocessed by the Robust Multichip Averaging (RMA) algorithm.[10] Next, identification of DEGs between the OS and the control group was carried out by 2-tailed Student's t-tests ($P < 0.05$, $|\log_2$ fold change (FC)$| \geq 2$).

Gene Functional Annotation

Transcription factor (TF) was identified by the functional annotation of DEGs using the TRANSFAC database (http://generegulation.com/pub/databases.html).[11] Furthermore, tumor suppressor genes (TSGs) and oncogenes were also screened through the database of TSG[12] and Tumor Associated Genes (TAG).[13]

Table 1. Primers for Quantitative Real-Time PCR.

Primer	Direction	Sequence (5'-3')
CENPE	Forward	AAGACCGAGCTTTCTTACAAGA
	Reverse	CTACAGTTTGCAGCGTAGAATC'
PRC1	Forward	CCTATTCTGAGTTTGCGAAGGA
	Reverse	TGATCAGGGCTTCTCAGGAC
TTK	Forward	TGGCCAACCTGCCTGTTT
	Reverse	AATGCATTCATTTGCTGAAGAAGA
PLK4	Forward	CCTTATCACCTCCTCCTTC
	Reverse	CCAAGTCCTTCATTTGTAACC
GAPDH	Forward	TGACAACTTTGGTATCGTGGAAGG
	Reverse	AGGCAGGGATGATGTTCTGGAGAG

KEGG Pathway and GO Functional Enrichment Analyses of DEGs

KEGG pathway[14] and GO functional[15] analyses were carried out to screen important pathways and the functional terms of the upregulated genes and downregulated genes, respectively, using Database for Annotation, Visualization and Integrated Discovery[16] with a P value of < 0.05.

PPI Network Construction

PPI network was built using search tool for the retrieval of interacting genes (STRING, version 9.1),[17] and visualized in Cytoscape. Only interactions with a combined score > 0.4 were hold. The proteins with higher degree of interactions were regarded as the hub nodes.

Real-Time PCR Verification of Key Genes

Four kinds of human OS cell lines, including MG63, HOS, Saos and U2OS cells, and the human mesenchymal stem cells were purchased from iCell Bioscience Inc. (Shanghai, China). Cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum, 1% streptomycin (100 μg/ml) and penicillin (100 U/ml) at 37°C and 5% CO_2.

Total RNA from cells were extracted from cells using Trizol reagent (Thermo Fisher Scientific Inc.). cDNA was synthesized from total RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc.). qPCR was then performed on ABI7500 with SYBR Green qPCR Master Mix (Thermo Fisher Scientific Inc.). GAPDH served as internal control. Primers are given in Table 1. The expression of gene was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Experimental data were represented as mean ± standard deviation. Comparisons were performed using 1-way analysis of variance with post-hoc test of Bonferroni test in Graphpad Prism. $P < 0.05$ was regarded as significant level.

Table 2. The List of 188 Differentially Expressed Genes.

Category	Genes
The upregulated genes	*ABCA1, ABHD17A, ADAMTS9, APLP1, ARL4C, ARRDC3, BRINP1, CACNB4, CC2D2A, CD163L1, CDR1, CHMP4C, CHST11, COL11A2, DAPK2, DCLK1, DDIT4, DOK6, DYNC1I1, EPHX1, EYA2, FAM27C, FAM49A, FBXO32, FOLR1, FOXRED2, FRMD4B, GATS, GHR, GSE1, HSPB8, IFNL3, INSR, IRX3, KAZALD1, KIAA1161, KLRC2, LAMA4, LARGE, LDLRAD4, LEF1, LOC100506127, LRRC15, MAFB, MAN1A1, MGMT, MIR181B1, MIR21, MTRF1 L, MTSS1, NBPF4, NCALD, NCAM1, NHSL1, NR1D1, NUPR1, PARK2, PBX1, PCDHGA4, PCDHGB1, PCDHGB4, PCDHGB6, PCDHGC3, PCOLCE2, PCSK5, PPFIBP2, PRB1, PSG1, RABGAP1 L, RDH10, RFTN2, RGCC, RGS3, SCUBE1, SEPT5, SERINC5, SESN3, SGCD, SLC4A8, SLC7A8, SNORA42, SNORD104, ST3GAL1, STEAP1B, SYBU, SYNPO, SYTL2, TENC1, TNFRSF19, TOX, TRPS1, TUBA4A, UNC5B, UST, UTY, WISP1, WLS, ZDHHC23, ZHX1, ZNF608*
The downregulated genes	*AKAP2, ALCAM, ANLN, ANXA3, BNC1, BTBD10, CD248, CENPE, CEP170, COL4A1, DAB2, DBF4, DIAPH3, DSE, FAM216A, FAM64A, FARP1, FSTL1, FZD2, GPR126, GPR176, HIST1H2BB, HIST1H4D, HLA-A, HLA-B, HMGB2, IGFBP7, KDM6A, KIF20B, KIF23, KLHL7, KNSTRN, KRT8, LPCAT2, MARCH4, MB21D2, MEST, METAP1, MICB, MTMR10, MYL9, NDFIP2, NMI, NRIP3, NRXN3, OSMR, PCDH10, PDLIM5, PFKP, PLK4, PPP2CB, PRC1, PRPS1, PTPLA, PTPN9, PTPRJ, PVRL3, RAB3IP, RAP1GDS1, RB1, RBPMS, RCN1, RHOJ, RNY5, RP2, SCRN1, SH3BGRL2, SHOC2, SIM2, SLC9A7, SNORD25, SNX25, SPTLC2, STARD4, STK10, STK32B, TAGLN, TMOD3, TPM1, TTK, TUBB6, UEVLD, UFSP2*

Results

DEGs in OS Cell Lines Compared With Control Cell

After analysis, 183 DEGs were identified, including 100 upregulated genes and 83 downregulated genes (Table 2). Among these DEGs, 5 upregulated TFs (*ZHX1*, *PBX1*, *NR1D1*, *MAFB* and *LEF1*) and 2 downregulated TFs (*SIM2* and *HMGB2*), as well as 8 upregulated TAGs (6 TSGs and 2 uncertain genes for tumor development) and 7 downregulated TSGs were further identified. The upregulated TSGs were *UNC5B*, *PARK2*, *MTSS1*, *MIR181B1*, *FBXO32* and *ADAMTS9*; the other upregulated genes were *MAFB* and *CDR*. The downregulated TSGs were *TPM1*, *RB1*, *PTPRJ*, *PPP2CB*, *PCDH10*, *IGFBP7* and *DAB2*.

Table 3. KEGG Pathway Enrichment Analysis of Differentially-Expressed Genes.

KEGG pathway	Gene counts	P-value
Upregulated		
Glycosaminoglycan biosynthesis–chondroitin sulfate	2	6.21×10^{-3}
Arrhythmogenic right ventricular cardiomyopathy	3	7.34×10^{-3}
Downregulated		
Cell adhesion molecules	5	2.90×10^{-4}
Pentose phosphate pathway	2	6.66×10^{-3}
Allograft rejection	2	1.20×10^{-2}
Graft vs. host disease	2	1.50×10^{-2}
Type I diabetes mellitus	2	1.64×10^{-2}
Cell cycle	3	1.86×10^{-2}
Autoimmune thyroid disease	2	2.35×10^{-2}
Natural killer cell mediated cytotoxicity	3	2.37×10^{-2}
Phagosome	3	3.21×10^{-2}
Viral myocarditis	2	4.06×10^{-2}
Adherens junction	2	4.38×10^{-2}
Antigen processing and presentation	2	4.71×10^{-2}

KEGG: Kyoto Encyclopedia of Genes and Genomes; Gene Counts: number of genes.

KEGG Pathway Enrichment Analysis for DEGs

Based on the criterion of $P < 0.05$, the upregulated DEGs were significantly enriched in "glycosaminoglycan biosynthesis-chondroitin sulfate" and "arrhythmogenic right ventricular cardiomyopathy." Meanwhile, the downregulated DEGs were significantly enriched in 12 pathways, such as "cell adhesion molecules" (CAMs), "pentose phosphate pathway," "allograft rejection," "type I diabetes mellitus" and "cell cycle." The results of significantly enriched pathways are presented in Table 3.

GO Term Results for DEGs

Table 4 shows the top 5 GO terms. The upregulated DEGs were significantly enriched in 92 biological process (BP) terms, 8 cellular component (CC) terms and 28 molecular function (MF) terms. The upregulated DEGs were significantly involved in "multicellular organismal metabolic process," "limb morphogenesis" and "hemophilic cell adhesion." These genes were mostly located on "golgi apparatus," "growth hormone receptor complex" and "collagen type XI." Most of these genes possess the molecular function of "insulin-like growth factor binding," "integrase activity" and "α-tubulin binding." Meanwhile, the downregulated DEGs were obviously enriched in 58 BP terms, 18 CC terms and 21 MF terms. The suppressed processes in OS included "Positive regulation of pathway-restricted SMAD protein phosphorylation," "Peptidyl-tyrosine dephosphorylation" and "Regulation of mitosis." The downregulated genes were mostly located on "Spindle," "major histocompatibility complex (MHC) class I protein complex" and "striated muscle thin filament." The primary functions of the downregulated DEGs were "WW domain binding,"

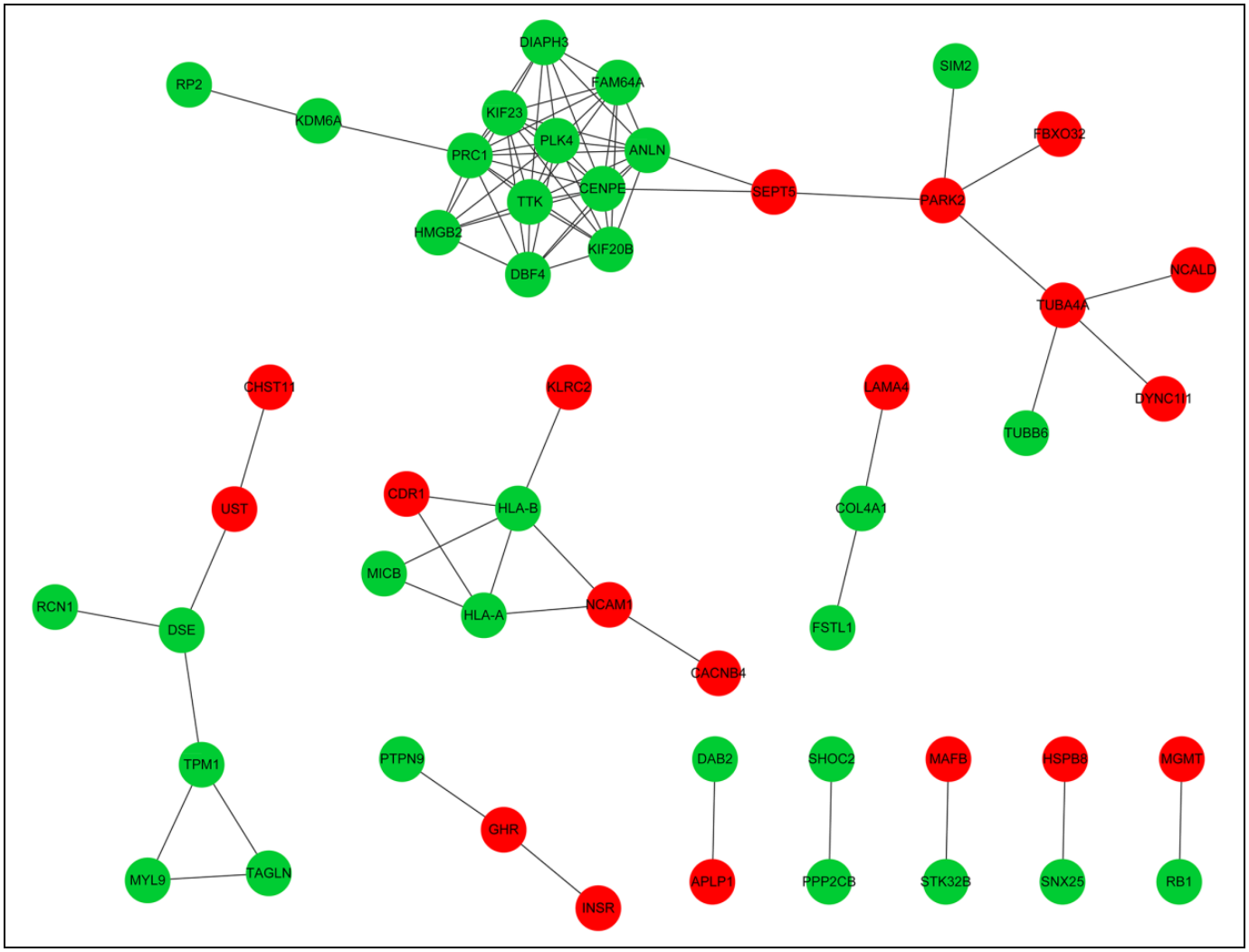

Figure 1. Protein-Protein Interaction (PPI) Network of Differentially Expressed Genes (DEGs) in Osteosarcoma. Red nodes represent upregulated DEGs; green nodes represent downregulated DEGs; gray lines stand for the interaction between 2 proteins. DEGs, differentially-expressed genes.

"polo kinase kinase activity" and "chondroitin-glucuronate 5-epimerase activity."

PPI Network of DEGs

Figure 1 shows the PPI network, which included 84 interactions and 51 nodes. For this network, the connectivity degree of each node was calculated and Table 5 shows the nodes with a degree of ≥ 2. According to the degrees of the nodes, centromere-associated protein E (CENPE), protein regulator of cytokinesis 1 (PRC1), phosphotyrosine picked threonine-protein kinase (TTK) and polo-like kinase 4 (PLK4) were selected as the hub nodes, as they interacted with more than10 nodes, indicating their crucial roles in the PPI network.

A total of 14 pathways were enriched by DEGs in PPI network, such as "glycosaminoglycan biosynthesis-chondroitin sulfate" and "natural killer cell mediated cytotoxicity" (Table 6). Furthermore, DEGs in the PPI network were also significantly enriched in 81 BP terms, 22 CC terms and 30 MF terms. The top 5 GO terms for each were listed in Table 7. For the BP terms, the dramatically enriched processes were mainly related with the "regulation of mitosis" and "cell division." For the CC terms, the significantly enriched components were mainly correlated with the "spindle" and "MHC class I protein complex." For the MF terms, the significantly enriched functions were mainly associated with "microtubule motor activity" and "chondroitin-glucuronate 5-epimerase activity."

Real-Time PCR Verification of the mRNA Expression of Key Genes

The CENPE, PRC1, TTK and PLK4 were selected as the hub nodes from PPI network. Based on the microarray results, these 4 genes were all significantly downregulated in OS cells. We performed real-time PCR to validate their expression in 4 kinds

Table 4. TOP 5 Significantly Enriched BP, CC and MF for Differentially-Expressed Genes.

GO ID	Term	Gene Counts	P-value
Upregulated			
GO:0044236_BP	Multicellular organismal metabolic process	5	5.24×10^{-4}
GO:0035108_BP	Limb morphogenesis	5	7.66×10^{-4}
GO:0007156_BP	Homophilic cell adhesion	5	8.18×10^{-4}
GO:0048703_BP	Embryonic viscerocranium morphogenesis	2	1.19×10^{-3}
GO:0009636_BP	Response to toxic substance	4	4.60×10^{-3}
GO:0005794_CC	Golgi apparatus	16	3.90×10^{-4}
GO:0070195_CC	Growth hormone receptor complex	1	5.16×10^{-3}
GO:0005592_CC	Collagen type XI	1	1.03×10^{-2}
GO:0005899_CC	Insulin receptor complex	1	1.54×10^{-2}
GO:0031982_CC	Vesicles	11	2.13×10^{-2}
GO:0005520_MF	Insulin-like growth factor binding	3	2.58×10^{-4}
GO:0008907_MF	Integrase activity	1	5.03×10^{-3}
GO:0005509_MF	Calcium ion binding	9	6.23×10^{-3}
GO:0043014_MF	α-tubulin binding	2	6.39×10^{-3}
GO:0042277_MF	Peptide binding	4	7.32×10^{-3}
Downregulated			
GO:0010862_BP	Positive regulation of pathway-restricted SMAD protein phosphorylation	3	2.42×10^{-4}
GO:0060391_BP	Positive regulation of SMAD protein import into nucleus	2	9.63×10^{-4}
GO:0035335_BP	Peptidyl-tyrosine dephosphorylation	3	1.32×10^{-3}
GO:0007088_BP	Regulation of mitosis	4	1.52×10^{-3}
GO:0051301_BP	Cell division	8	2.20×10^{-3}
GO:0005819_CC	Spindle	9	9.35×10^{-7}
GO:0042612_CC	MHC class I protein complex	2	9.44×10^{-4}
GO:0005865_CC	Striated muscle thin filament	2	2.03×10^{-3}
GO:0005900_CC	Oncostatin-M receptor complex	1	1.26×10^{-2}
GO:0005945_CC	6-phosphofructokinase complex	1	1.26×10^{-2}
GO:0050699_MF	WW domain binding	2	4.91×10^{-3}
GO:0042801_MF	Polo kinase kinase activity	1	5.02×10^{-3}
GO:0047757_MF	Chondroitin-glucuronate 5-epimerase activity	1	5.02×10^{-3}
GO:0008017_MF	Microtubule binding	4	6.70×10^{-3}
GO:0042605_MF	Peptide antigen binding	2	8.64×10^{-3}

GO: Gene Ontology; BP: biological process; CC: cellular component; MF: molecular function; Gene Counts: number of genes.

Table 5. Differentially-Expressed Genes With >2 Degrees of Connectivity in the Protein-Protein Interaction Network.

Gene	Degrees
CENPE	11
PRC1	11
TTK	10
KIF23	10
ANLN	10
PLK4	10
FAM64A	8
DBF4	8
KIF20B	8
DIAPH3	7
HMGB2	6
HLA-B	5
PARK2	4
HLA-A	4
TUBA4A	4
SEPT5	3
DSE	3
TPM1	3
NCAM1	3
CDR1	2
UST	2
KDM6A	2
COL4A1	2
MYL9	2
TAGLN	2
GHR	2
MICB	2

Table 6. KEGG Pathway Enrichment Analysis of Differentially-Expressed Genes in the Protein-Protein Interaction Network.

KEGG Pathway	Gene counts	P-value
Glycosaminoglycan biosynthesis-chondroitin sulfate	3	1.12×10^{-4}
Phagosome	5	4.76×10^{-4}
Natural killer cell mediated cytotoxicity	4	2.77×10^{-3}
Antigen processing and presentation	3	4.38×10^{-3}
Small cell lung cancer	3	5.99×10^{-3}
Allograft rejection	2	1.14×10^{-2}
Graft-vs.-host disease	2	1.39×10^{-2}
Type I diabetes mellitus	2	1.52×10^{-2}
Cell cycle	3	1.67×10^{-2}
Cell adhesion molecules	3	2.02×10^{-2}
Autoimmune thyroid disease	2	2.18×10^{-2}
Pathogenic *Escherichia coli* infection	2	2.51×10^{-2}
Viral myocarditis	2	3.79×10^{-2}
Cardiac muscle contraction	2	4.51×10^{-2}

KEGG, Kyoto Encyclopedia of Genes and Genomes; Gene Counts, number of genes.

of human OS cell lines, including MG63, HOS, Saos and U2OS cells. As shown in Figure 2, the expression of *CENPE*, *PRC1* and *TTK* were all significantly decreased in all 4 OS cells

Table 7. TOP 5 Significantly Enriched BP, CC and MF Terms for Differentially-Expressed Genes in the Protein-Protein Interaction Network.

GO ID	Term	Gene Counts	P-value
GO:0007088_BP	Regulation of mitosis	5	2.40×10^{-5}
GO:0051301_BP	Cell division	8	1.93×10^{-4}
GO:0002480_BP	Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent	2	3.79×10^{-4}
GO:0006266_BP	DNA ligation	2	5.76×10^{-4}
GO:0030208_BP	Dermatan sulfate biosynthetic process	2	5.76×10^{-4}
GO:0007080_BP	Mitotic metaphase plate congression	2	8.14×10^{-4}
GO:0005819_CC	Spindle	8	4.68×10^{-7}
GO:0042612_CC	MHC class I protein complex	2	4.38×10^{-4}
GO:0000139_CC	Golgi membrane	7	1.00×10^{-3}
GO:0070195_CC	Growth hormone receptor complex	1	2.87×10^{-3}
GO:0001725_CC	Stress fiber	2	6.78×10^{-3}
GO:0003777_MF	Microtubule motor activity	4	9.65×10^{-5}
GO:0047757_MF	Chondroitin-glucuronate 5-epimerase activity	1	3.13×10^{-3}
GO:0005200_MF	Structural constituent of cytoskeleton	3	3.16×10^{-3}
GO:0042605_MF	Peptide antigen binding	2	3.44×10^{-3}
GO:0042169_MF	SH2 domain binding	2	4.48×10^{-3}

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; Gene counts, number of genes.

compared with the human mesenchymal stem cells ($P < 0.05$). The expression of PLK4 was significantly decreased in HOS and U2OS cells ($P < 0.05$), and its expression levels in MG63 and Saos were also decreased in MG63 and Saos cells, though not reach the significant level ($P > 0.05$).

Discussion

In the current study, 100 upregulated and 83 downregulated DEGs in OS compared with control groups were obtained. Pathway enrichment analysis revealed that the upregulated genes were significantly enriched in "Glycosaminoglycan biosynthesis-chondroitin sulfate" and "arrhythmogenic right ventricular cardiomyopathy" and the downregulated genes were significantly enriched in 12 pathways, such as "CAMs," and "pentose phosphate pathway." GO-BP analysis suggested the upregulated genes were significantly enriched in 92 BP terms, such as "multicellular organismal metabolic process," and the downregulated genes were significantly enriched "positive regulation of pathway-restricted SMAD protein phosphorylation process." Furthermore, CENPE, PRC1, TTK and PLK4 were selected as the hub nodes in PPI network. DEGs in this network were significantly enriched in the "glycosaminoglycan biosynthesis-chondroitin sulfate" pathway, "regulation of mitosis process" and "microtubule motor activity" functions.

Our results showed that the upregulated DEGs and the DEGs in PPI network were obviously enriched in the "glycosaminoglycan biosynthesis-chondroitin sulfate" pathway and GO-BP of "multicellular organismal metabolic process." The downregulated DEGs were significantly enriched in "CAMs" pathway and GO-BP of "positive regulation of pathway-restricted SMAD protein phosphorylation process." Glycosaminoglycans are part of proteoglycans and defects in glycosaminoglycan synthesis could lead bone diseases.[18] Besides, alteration of glycosaminoglycan in extracellular matrix was reported to be involved in cell proliferation and differentiation.[19] Previous evidence has shown that glycosaminoglycan biosynthesis-chondroitin sulfate has relevance with growth and proliferation in human OS cells of MG63 and Saos cells.[19,20] A previous study indicated that CAMs involved in the process of the metastasis of OS[21] and CAM1 was found to be a diagnostic marker for OS.[22] The SMAD signaling pathway plays role in some cancer cellular processes, e.g. proliferation and apoptosis.[23] In addition, the present results showed that the regulation of mitosis biology process and microtubule motor activity molecular function was significantly enriched in the PPI network. Studies indicated that microtubule motor activity involved in the mitosis of OS cells and could promote OS cell proliferation.[24,25] Therefore, our present study is in line with the previous study, suggesting that glycosaminoglycan biosynthesis-chondroitin sulfate pathway, regulation of mitosis biology process and microtubule motor activity may play critical roles in the progression of OS.

In the PPI network, CENPE was the top node and interacted with 11 proteins, including PRC1. These 2 genes were closely associated with the biological process of cell division. CENPE is a mitotic motor whose inactivation disrupts spindle checkpoint function.[26,27] Furthermore, the incidences of spontaneous lymphomas and lung tumors are elevated in mice with reduced levels of CENPE, which agrees with its decreased level in OS group, suggesting that low CENPE level may facilitate the progression of OS.[28] In another bioinformatics-based study, CENPE was also identified as downregulated gene and a high-degree node in PPI network of osteosarcoma.[29] However, CENPE was reported to be upregulated in several types of cancer, including lung cancer, esophageal cancer and breast cancer.[30-32] It is reported that PRC1 plays key roles in the central spindle and cytokinesis formation.[33] A previous study testified that PRC1 may involve in growth of tumor cell and may be a possible target for anti-breast cancer drugs.[34] However, it has not been reported whether PRC1 could be a target for OS treatment. Therefore, we speculate that the interaction between PRC1 and CENPE may be critical for the progression of OS.

The PPI network in the current study also showed that TTK was a hub node with a higher degree that directly interacted with PLK4. TTK plays significant roles in centrosome

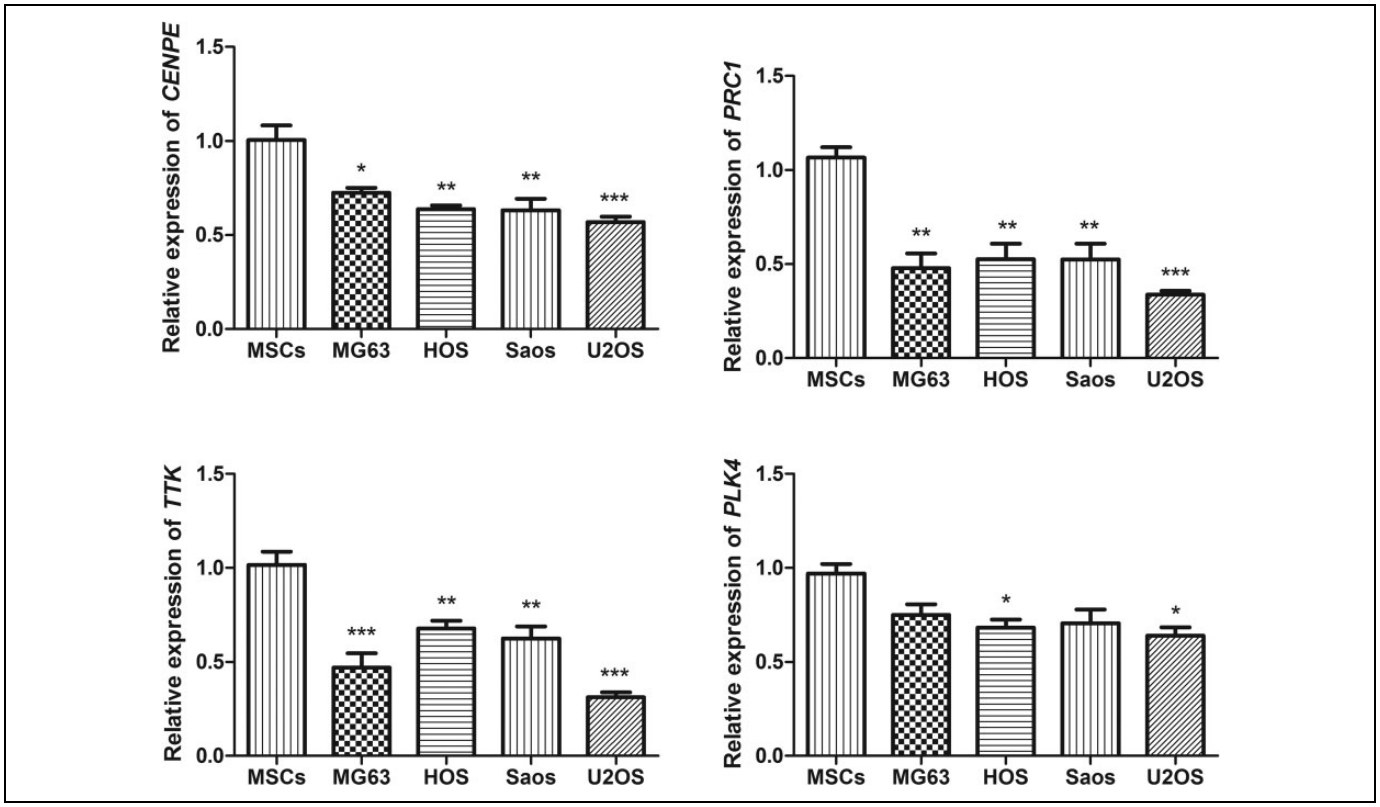

Figure 2. Real-time PCR analysis of CENPE, PRC1, TTK and PLK4 in 4 kinds of osteosarcoma cells, including MG63, HOS, Saos and U2OS cells and human mesenchymal stem cells (MSCs). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the human mesenchymal stem cells.

duplication, the proper execution of mitosis and tumor cell proliferation. In addition, significant overexpression of TTK is found in some human tumors.[35] Caldarelli et al showed that selective TTK inhibitors can inhibit U2OS cells proliferation by promoting mitotic override.[36] Moreover, TTK has been observed to affect the NF-κB signaling pathway, and NF-κB activation is common in carcinomas, mainly promoted by inflammatory cytokines within the tumor microenvironment.[37] PLK4, a member of the polo-like kinase family, is a critical regulator of centriolar duplication.[38] *In vitro*, evidence has demonstrated that the depletion of PLK4 with small interfering RNA results in the decrease of U2OS cells proliferation.[37] In addition, the overexpression of PLK4 contributes to amplification of centrosomes, while the deletion of it decreases the number of centriole in U2OS cells. Furthermore, PLK4 is directly targeted by NF-κB, which is absolutely critical for cell proliferation in U2OS cells. For OS, Tang et al. indicated that the combined use of NF-κB inhibitors and chemotherapy drugs can improve the *in vitro* and *in vivo* effects of the chemotherapy drugs.[4] Therefore, we considered that the interaction between TTK and PLK4 may involve in the centrosome duplication and the growth of OS cells through the activation of NF-κB pathway in OS.

However, this study has some limitations. First, because of lacking OS patients, the expression levels of key genes including CENPE, PRC1, TTK, and PLK4 were not validated in OS tissue or serum samples. Second, the interactions among proteins as well as the molecular mechanism of these key proteins in regulating OS development warrant further investigation.

In conclusion, "glycosaminoglycan biosynthesis-chondroitin sulfate" pathway, "microtubule motor activity," and the "regulation of mitosis biology process" may be critical for OS progression. The interaction between PRC1 and CENPE may be important for OS progression. The interaction between PLK4 and TTK may be involved in the centrosome duplication and the growth of OS cells through NF-κB pathway activation in OS. CENPE, PRC1, TTK, and PLK4 may be biomarkers for the diagnosis and treatment of OS. However, further studies with a larger sample size are warranted to confirm these results.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Statement

Not applicable. Our study did not contain human or animal trials.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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