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ORIGINAL ARTICLE

Identification of Differentially Expressed Genes under the Regulation of Transcription Factors in Osteosarcoma

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Abstract

The present study was to investigate and identify the differentially expressed genes (DEGs) in the transcriptional regulatory network of osteosarcoma (OS). The gene expression dataset from Gene Expression Omnibus (GEO) datasets was downloaded. DEGs were identified and their functional annotation was also conducted. In addition, differentially expressed transcription factors (TFs) and the regulatory genes were identified. The electronic validation was used to verify the expression of selected genes. The integrated analysis led to 932 DEGs. The results of functional annotation indicated that these DEGs significantly enriched in the p53 signaling pathway, Jak-STAT signaling pathway and Wnt signaling pathway. ZNF354C, NFIC, NFATC2, SP2, FOXO3, EGR1, ZEB1, RREB1, EGR2 and SRF were covered by most TFs. The expression levels of NFIC and EGR2 in electronic validation were compatible with our bio-informatics result. In conclusion, the deregulation of these genes may provide valuable information in understanding the underlying molecular mechanism in the OS.

Keywords Osteosarcoma · Microarray dataset · Differentialy expressed genes · Transcription factors

Abbreviations

DEGs	Differentially expression genes
EGR1	Early growth response 1
EGR2	Early growth response 2
FDR	False discovery rate
FOXO3	Forkhead box O3
GEO	Gene expression omnibus
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
NFIC	Nuclear factor I C
NFATC2	Nuclear factor of activated T-cells 2
OS	Osteosarcoma
PWM	Position weight matrix
RREB1	Ras responsive element binding protein 1
SRF	Serum response factor
SP2	Sp2 transcription factor
TFs	Transcription factors
ZEB1	Zinc finger E-box binding homeobox 1
ZNF354C	Zinc finger protein 354C

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Introduction

Osteosarcoma (OS), a tumour of mesenchymal origin, is a common type of primary bone cancer with highly metastatic potential [1]. It occurs frequently in adolescents, followed by a second incidence peak among older individuals (age > 60) [2, 3]. The traditional treatment strategy of OS is completely removing tumour by aggressive chemotherapy and wide excision [4]. Although some treatments including chemotherapy, radiotherapy and surgery have been performed, patients with recurrent or metastatic OS remain have poor prognosis [5].

Up to now, the exact mechanism of OS is unclear. It is reported that the disease course of the OS patients is variable, and the pathogenesis and prognostic factors still poorly understood [6]. Therefore, the identification of new molecules as favorable drug targets to provide novel therapeutic strategies is crucial for improving clinical outcome of patients suffering OS. Many researchers have found several genes were involved in the pathogenesis of osteosarcoma. SEE-HYOUNG PARK et al. reported FOXO3 is a promising candidate for the development of osteosarcoma therapy, as these therapies may

Table 1 Characteristics of nine datasets in OS

GEO ID	Sample count (case:control)	Platform	Sample source	Tissue
GSE11414	4:2	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array	in vitro	bone
GSE12865	12:2	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array	in vivo	bone
GSE14359	10:2	GPL96 [HG-U133A] Affymetrix Human Genome U133A Array	in vivo	bone, lung
GSE32964	35:1	GPL6947 Illumina HumanHT-12 V3.0 expression beadchip	in vivo	bone
GSE36001	20:6	GPL6102 Illumina human-6 v2.0 expression beadchip	in vitro	bone
GSE42352	103:15	GPL10295 Illumina human-6 v2.0 expression beadchip (using nuIDs as identifier)	in vivo/in vitro	bone
GSE42572	7:5	GPL13376 Illumina HumanWG-6 v2.0 expression beadchip	in vivo	bone
GSE56001	6:6	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip	in vitro	bone
GSE70414	5:1	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	in vitro	bone

sensitize osteosarcoma cells to FOXO3-mediated apoptosis and suppress tumorigenesis. Yukihiro Matsunoshita et al. found that chemotherapy can prevent osteosarcoma cell invasion by down-regulation of urokinase plasminogen activity via up-regulation of EGR1 during chemotherapy periods. Shen A et al. reported the overexpression of ZEB1 in osteosarcoma may be related to the carcinogenesis and development as well as metastasis and invasion of osteosarcoma. Human TFs regulate thousands of downstream genes via binding to specific DNA sequences in the promoter region of genes and TFs regulatory networks are foundations to biological systems [7].

In this study, we performed an integrated analysis of OS gene expression data to identify DEGs between OS and normal tissues. Making use of TRANSFAC and the integrated analysis of gene expression data, we obtained a set of differentially expressed TFs regulating gene expression in the development of OS pathogenesis. TFs regulatory networks were also constructed for a systematic understanding of disease progression at the molecular level. The GSE 16088 dataset was used to verify the expression of selected genes. Identification of crucial differentially expressed genes under the regulation of TFs may provide new potential therapeutic targets for the OS.

Materials and Methods

Datasets of OS

Gene expression profiles of OS were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) [8]. The following keywords were used "Osteosarcoma, OS" [MeSH Terms] OR Osteosarcoma, OS [All Fields] AND "*Homo sapiens*" [porgn] AND "gse" [Filter]. All selected datasets were genome-wide expression data of OS group and/or normal group and downloaded for integrated analysis.

Table 2 Top 10 up- and down-regulated DEGs in OS

ID	Symbol	Log FC	FDR	ID	Symbol	Log FC	FDR
1404	HAPLN1	8.99E + 00	2.4724E − 38	202018	TAPT1	-8.40E-01	2.35113E-26
55220	KLHDC8A	5.59E + 00	7.22112E-32	80333	KCNIP4	-2.82E + 00	6.40665E-24
2118	ETV4	4.06E + 00	3.57843E-31	51115	FAM82B	-8.98E-01	4.02609E-23
200879	LIPH	7.96E + 00	1.94466E-30	9060	PAPSS2	-3.16E + 00	1.31188E-21
196410	METTL7B	4.22E + 00	5.27141E-30	26034	IPCEF1	−4.45E + 00	5.25893E-21
84069	PLEKHN1	4.44E + 00	2.18739E-28	201627	FAM116A	−1.23E + 00	5.25893E-21
27113	BBC3	2.56E + 00	6.32646E-28	54537	FAM35A	−9.08E-01	6.13967E-21
2561	GABRB2	7.02E + 00	9.45656E-28	22925	PLA2R1	−3.74E + 00	7.92896E-21
219699	UNC5B	2.24E + 00	1.0945E-27	9759	HDAC4	−1.22E + 00	2.49564E-20
8974	P4HA2	2.03E + 00	2.18012E-27	83693	HSDL1	−1.02E + 00	2.78091E-20

Table 3 Significantly enriched gene ontology terms of DEGs

GO ID	GO term	No.of genes	P-vaule
Biological process			
GO:1901360	organic cyclic compound metabolic process	75	3.18E-04
GO:0001501	skeletal system development	3	3.64E-04
GO:0010882	regulation of cardiac muscle contraction by calcium ion signaling	3	4.38E-04
Cellular component			
GO:0005634	nucleus	125	6.40E-04

Identification of DEGs in OS

The raw data were preprocessed by background correction and normalization. The limma package in R was used to analyze the differential expression between the OS and the normal tissues by t-test. The p value and false discovery rate (FDR) were calculated and genes with FDR < 0.01 were seen as DEGs in our study.

Functional Annotation of DEGs

Biological functions and biological pathways of the DEGs in OS were interpreted by gene ontology (GO) enrichment analysis by GO-rilla (http://cbl-gorilla.cs.technion.ac.il/) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis by GeneCoDis3 (http://genecodis.cnb.csic.es/analysis). It was considered to be statistically significant when $p < 0.001$.

Construction of Transcriptional Regulatory Networks

Sequence-specific TFs are important effectors of eukaryotic gene control. To understand the regulatory mechanisms between DEGs and TFs in OS, we searched TRANSFAC to find genomic binding sites and DNA binding site sequence profiles

Table 4 Top 15 most significantly enriched Kyoto encyclopedia of genes and genomes pathways of DEGs

KEGG ID	KEGG term	Count	FDR	Genes
hsa05200	Pathways in cancer	31	1.43E-07	MET,BCL2L1,FGF1,FGF10,KIT,TGFA,GLI3,ITGA3, PIK3R2,E2F1,CCND1,ITGA2,PDGFA,RUNX1, CDKN2B,LAMB3,CDKN1A,TRAF6,PML,BID, TGFB1,JUN,BCL2,BAX,SMAD4,RXRG,CDKN2A, RARA,DVL1,FN1,FGFR2
hsa04360	Axon guidance	17	5.22E-06	NGEF,SEMA4C,MET,SEMA6B,LIMK1,EPHB1, PLXNA3,UNC5B,SEMA3D,SLIT1,SEMA3F,RGS3, EPHB3,FYN,EPHA2,EFNA3,EPHA3
hsa05222	Small cell lung cancer	12	9.21E-05	BCL2L1,ITGA3,PIK3R2,E2F1,CCND1,ITGA2,CDKN2B, LAMB3,TRAF6,BCL2,RXRG,FN1
hsa04115	p53 signaling pathway	11	1.05E-04	DDB2,SHISA5,TNFRSF10B,CCND2,CCND1,CDKN1A, BID,BBC3,BAX,CCND3,CDKN2A
hsa04510	Focal adhesion	18	2.33E-04	MET,RELN,IBSP,CCND2,ITGA3,BCAR1,PIK3R2,CCND1, ITGA2,PDGFA,LAMB3,JUN,VASP,BCL2,FYN,CCND3, ACTN4,FN1
hsa05212	Pancreatic cancer	5	4.47E-04	E2F1,CCND1,TGFB1,SMAD4,CDKN2A
hsa05162	Measles	5	4.47E-04	TNFRSF10B,CCND2,CCND1,BBC3,CCND3
hsa00350	Tyrosine metabolism	5	5.03E-04	ADH1A,ADH5,ALDH1A3,AOX1,ADH1B
hsa00982	Drug metabolism -cytochrome P450	5	5.03E-04	ADH1A,ADH5,ALDH1A3,AOX1,ADH1B
hsa04110	Cell cycle	13	5.35E-04	CCND2,E2F1,CCND1,CDKN2B,CDKN1A,TGFB1,MAD2L2, CDC45,PKMYT1,SMAD4,CCND3,CDC14B,CDKN2A
hsa05220	Chronic myeloid leukemia	6	5.42E-04	E2F1,CCND1,CDKN1A,TGFB1,SMAD4,CDKN2A
hsa04630	Jak-STAT signaling pathway	3	5.42E-04	E2F1,CCND1,CDKN1A,TGFB1,SMAD4,CDKN2A
hsa04310	Wnt signaling pathway	3	5.47E-04	CCND2,CCND1,CCND3
hsa05218	Melanoma	9	9.31E-04	MET,FGF1,FGF10,PIK3R2,E2F1,CCND1,PDGFA, CDKN1A,CDKN2A
hsa00010	Glycolysis	4	9.58E-04	ADH1A,ADH5,ALDH1A3,ADH1B

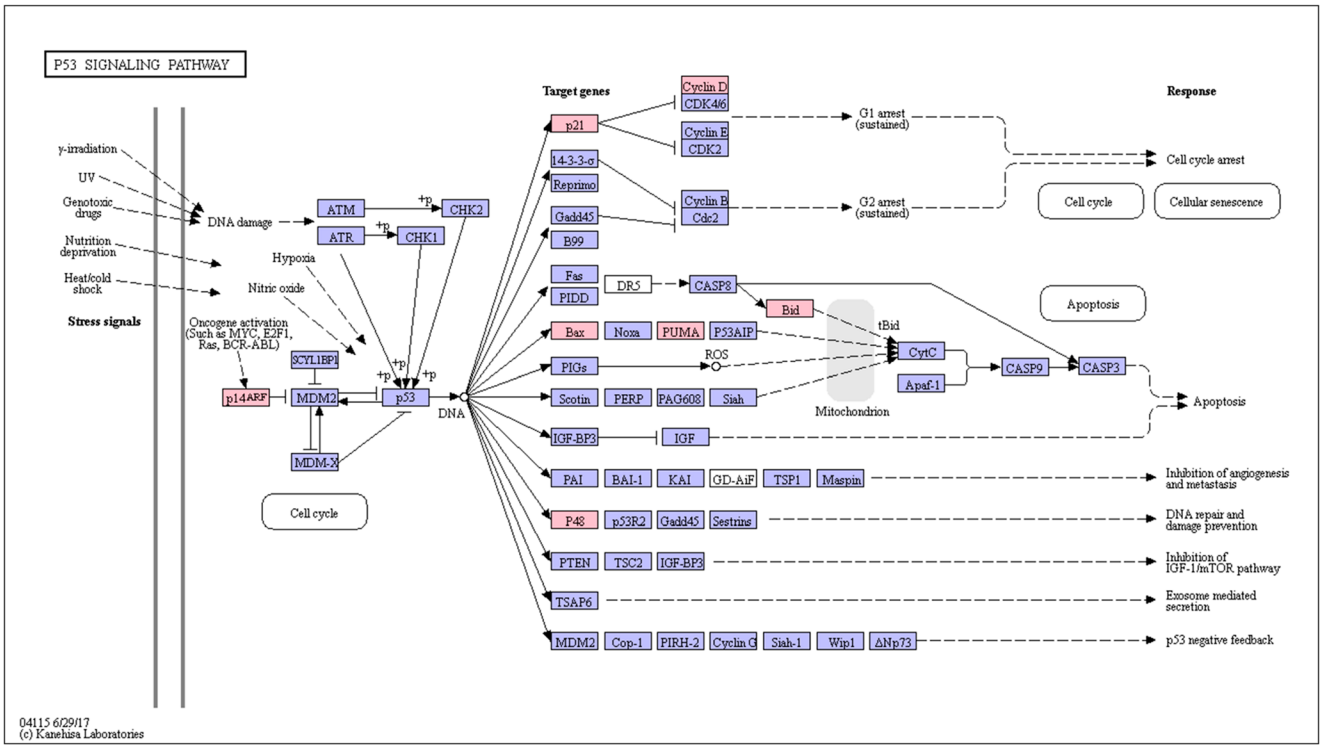

Fig. 1 Significantly enriched p53 signaling pathway. The colored rectangles were represented genes that enriched in p53 signaling pathway

for DEGs coded TFs and their targeted genes, and scanned gene promoter by TRANSFAC position weight matrix (PWM) to identify DEGs [9]. The transcriptional regulatory networks were established by Cytoscape.

Validation of DEGs in the Database of GEO

The GSE33382 database (14 cases and 6 normal controls) was used to validate the expression of selected miRNAs and targeted genes. We compared the expression levels of genes between OS cases and adjacent non-tumor controls and the difference of expression levels were displayed by box-plots.

ROC Analysis

In order to access the diagnostic value of DEGs for OS, the "pROC" package was used to calculate ROC, and the area under the ROC curve (AUC) was further calculated. When AUC value was greater than 0.6, the DEGs were considered

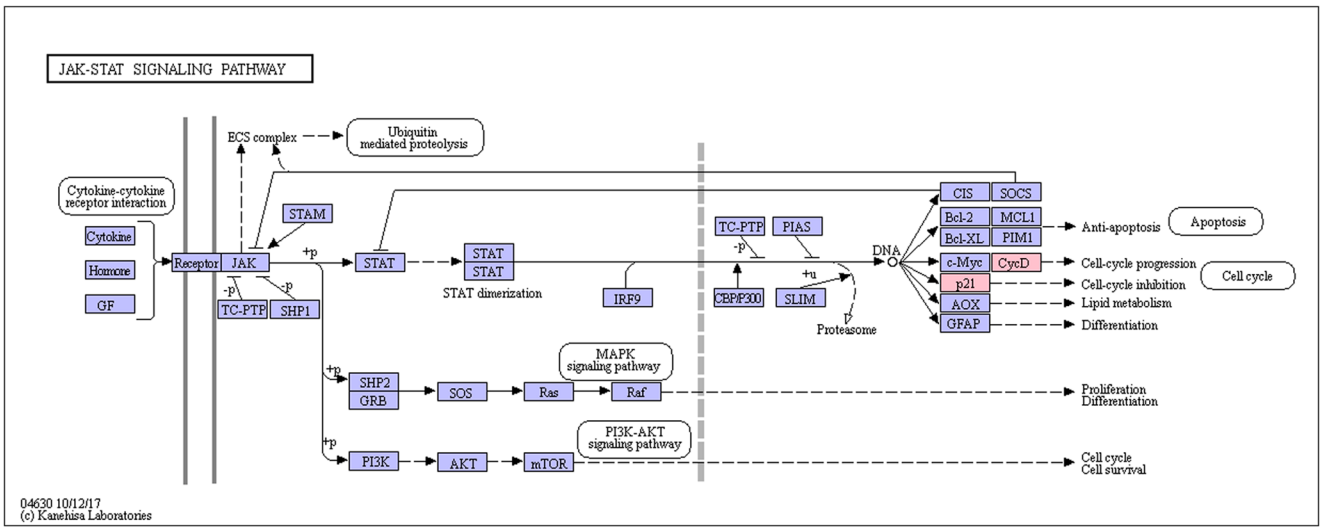

Fig. 2 Significantly enriched Jak-STAT signaling pathway. The colored rectangles were represented genes that enriched in Jak-STAT signaling pathway

capable of distinguishing patients with OS and normal controls with excellent specificity and sensitivity.

Results

Gene Expression Profiles in OS

In this study, 9 datasets of OS were included, and the detailed information of datasets was showed in Table 1. Totally, 202 cases of OS and 40 controls of normal tissues were included in the integrated analysis. Nine hundred thirty-two genes (475 up-regulated and 457 down-regulated) were regarded as DEGs under the selection criteria of FDR < 0.01. The top 10 up- and down-regulated DEGs were presented in Table 2.

Annotated Functions of DEGs

The functional analysis of DEGs based on GO annotations and KEGG pathway analysis manifested that these DEGs were significantly enriched in organic cyclic compound metabolic process, nucleus, pathways in cancer, p53 signaling pathway, focal adhesion, chronic myeloid leukemia, Jak-STAT signaling pathway, Wnt signaling pathway and melanoma. Table 3 was the GO annotations of the identified DEGs. The top 15 DEGs of KEGG pathway analysis was listed in Table 4. The KEGG map of p53 signaling pathway, Jak-STAT signaling pathway and Wnt signaling pathway was shown in Figs. 1, 2, and 3.

Transcriptions Regulatory Networks

The regulatory networks between DEGs and TFs were created. Based on TRANSFAC, 46 differentially expressed TFs were identified. Regulatory networks consisted of 819 TF-target interactions between 46 TFs and 509 DEGs in the context of OS (Fig. 4). The top 10 TFs (all down-regulated) covering the most downstream DEGs were regarded as crucial TFs involved in the pathology of OS and listed in Table 5, including zinc finger protein 354C (ZNF354C), nuclear factor I C (NFIC), nuclear factor of activated T-cells 2 (NFATC2), Sp2 transcription factor (SP2), forkhead box O3 (FOXO3), early growth response 1 (EGR1), zinc finger E-box binding homeobox 1 (ZEB1), ras

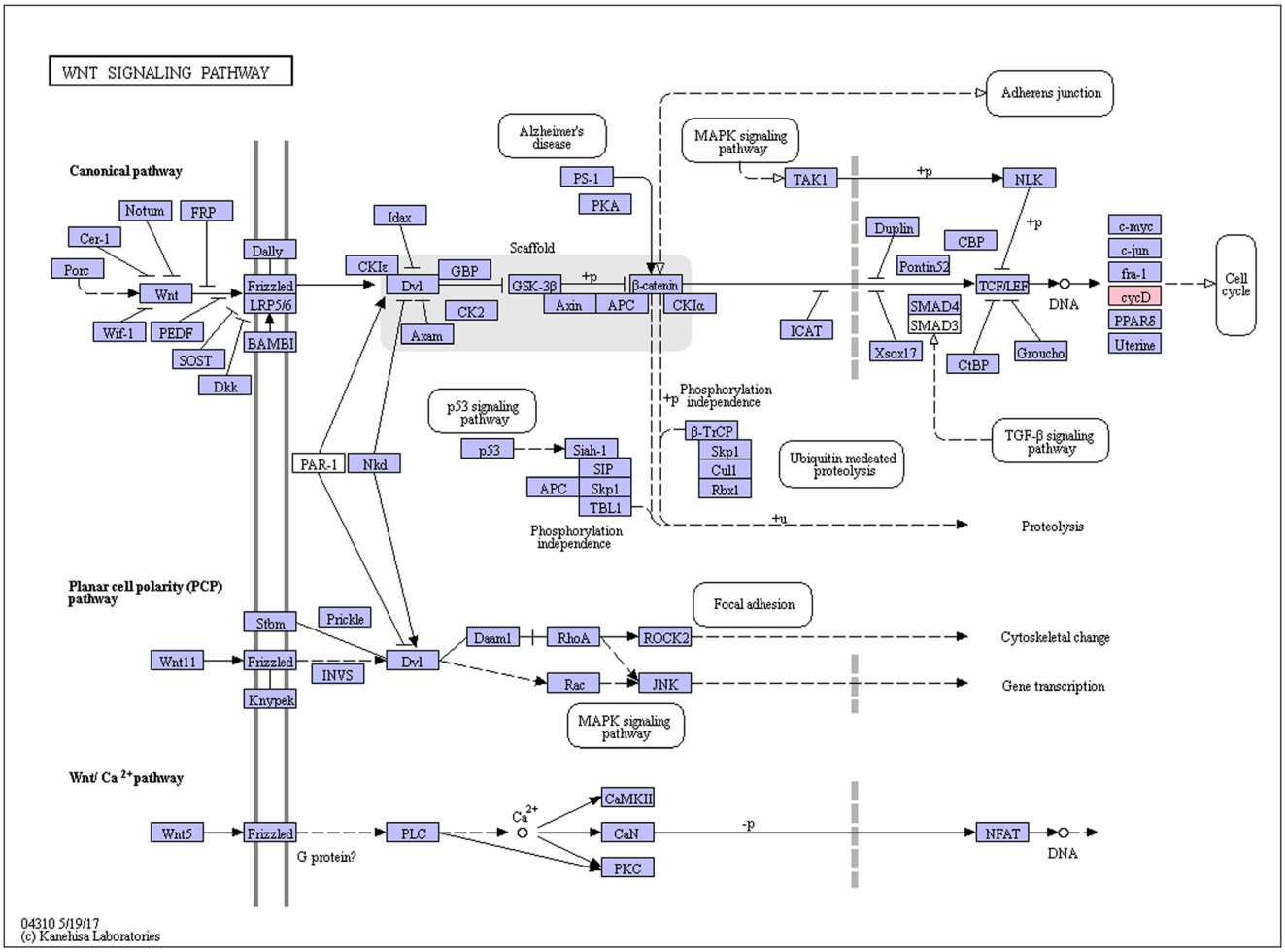

Fig. 3 Significantly enriched Wnt signaling pathway. The colored rectangles were represented genes that enriched in Wnt signaling pathway

responsive element binding protein 1 (RREB1), early growth response 2 (EGR2) and serum response factor (SRF).

Validation the Expression of Genes

In this study, six down-regulated genes (ZNF354C, NFIC, EGR1, RREB1, SRF and EGR2) in OS were selected to perform the expression validation (Fig. 5). Different expression levels of them between OS and non-tumor tissues were analyzed and depicted through box-plots. These box-plots were displayed by median and inter-quartile range visually. The expression levels of ZNF354C, NFIC, EGR1, RREB1, SRF and EGR2 were significantly down-regulated in the disease group compared to the control group, which was consistent with our bio-informatics analysis result.

ROC Curve Analysis

ROC curve analyses and the AUC were used to assess the discriminatory ability of six DEGs among 202 OS and 40 normal control derived from GEO. The AUC of all these six DEGs including ZNF354C (0.664), NFIC (0.920), EGR1 (0.671), RREB1 (0.835), SRF (0.813) and EGR2 (0.722) was more than 0.6 (Fig. 6) which have great diagnostic value for OS. For OS diagnosis, the specificity and sensitivity of ZNF354C was 0.667 and 0.600; the specificity and sensitivity of NFIC was 0.821 and 1.000; the specificity and sensitivity of EGR1 was 0.869 and 0.600, the specificity and sensitivity of RREB1 was 0.893 and 0.667, the specificity and sensitivity of SRF was 0.726 and 0.867, the specificity and sensitivity of EGR2 was 0.714 and 0.733, respectively.

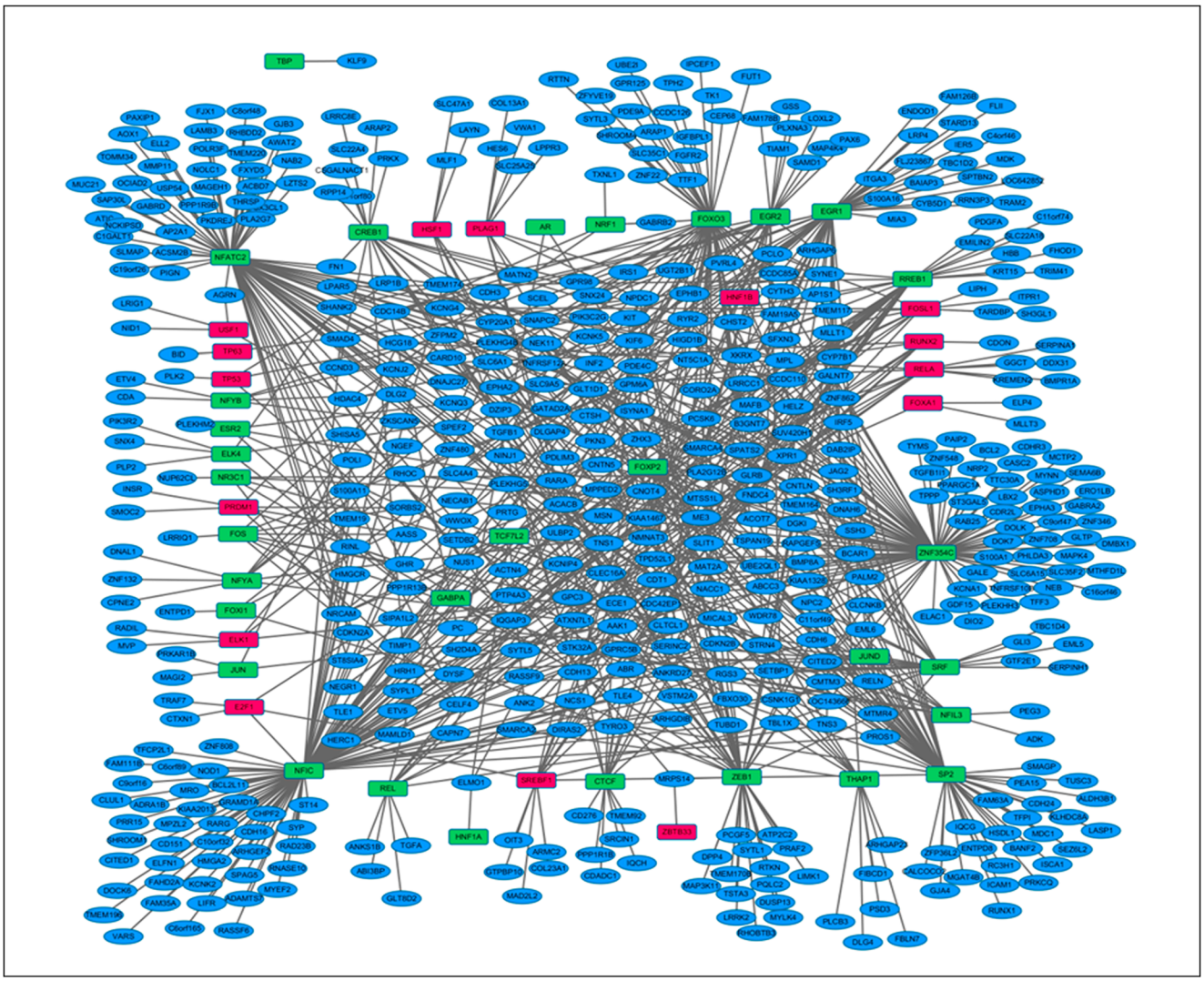

Fig. 4 The established transcriptional regulatory network of OS. Red- and green-color nodes represent up- and down-regulated TFs, respectively. Blue nodes denote DEGs predicted to interact with the corresponding TFs

Table 5 The top 10 TFs covering the most downstream DEGs in OS

Transcription factor	logFC	Up/down	Count	Genes
ZNF354C	−1.42E + 00	down	96	ASPHD1,GDF15,GPRC5B,CDT1,SEMA6B,SFXN3,FAM19A5,ELAC1,TSPAN19,ACTN4, C9orf47,RASSF9,ZNF480,ACOT7,NPC2,CCDC110,TYMS,NEB,PIK3C2G,PLA2G12B, CNTN5,GALNT7,CITED2,GABRA2,KCNA1,BCL2,HRH1,MPL,SLC4A4,SLIT1, SUV420H1,CMTM3,NT5C1A,RAPGEF5,NECAB1,PPARGC1A,NACC1, LOC143666,ZNF548,SCEL,GLT1D1,MCTP2,PCSK6,TGFB1I1,CDR2L, TMEM164,PHLDA3,DOLK,TPPP,SLC6A15,UGT2B11,ZNF346,TMEM174,RHOC, VSTM2A,B3GNT7,MYNN,PAIP2,MAFB,ABCC3,C11orf49,CDHR3,DIO2,CYTH3, MTHFD1L,DMBX1,DOK7,HIGD1B, XKRX,EPHA3,GALE,PLEKHH3,CARD10,RAB25,TFF3,LPAR5,NRP2, LBX2,PKN3,SLC35F2,CASC2,PLEKHG4B,C16orf46,RYR2,SIPA1L2,S100A1, ST3GAL5,ERO1LB,TPD52L1,MAPK4,BMP8A,TNFRSF10B,TTC30A, TNFRSF12A,GLTP,ZNF708
NFIC	−6.58E-01	down	95	BCL2L11,SMAD4,NT5C1A,ST8SIA4,TLE4,SYP,HMGCR,KCNK2,ISYNA1,PRR15, HMGA2,DOCK6,TSPAN19,SPAG5,C10orf32,ADRA1B,RELN,FAHD2A,PDLIM3,HERC1, NMNAT3,NEGR1,C6orf89,SYTL5,HIGD1B,RAD23B,RNASE10,RINL,CHPF2,DYSF, SHROOM1,CDT1,ARHGDIB,TFCP2L1,RGS3,PROS1,RASSF6,DAB2IP,TMEM196, BCAR1,MPZL2,POLI,LIFR,CDH16,ZNF862,FAM111B,ARHGEF2,MYEF2,EML6, KIAA2013,PLA2G12B,CITED1,PRTG,SSH3,MRO,VARS,ADAMTS7,TMEM174, CD151,IRF5,SNX24,SLIT1,TLE1,KIAA1467,MPPED2,DLGAP4,KCNJ2,ELFN1, CDKN2A,FBXO30,S100A11,RARG,HRH1,TUBD1,ST14,GPRC5B,ETV5,AAK1, E2F1,CDKN2B,C9orf16,GRAMD1A,SLC6A1,ZNF808,VSTM2A,FAM35A,TMEM19, CLUL1,CYP20A1,NOD1,LRRCC1,SYPL1,NUS1,C6orf165,UGT2B11
NFATC2	−1.74E + 00	down	84	TMEM174,PLA2G7,TNS1,DNAJC27,ZNF480,CDKN2A,MAGEH1,MTSS1L,MAFB, MUC21,ELL2,ST8SIA4,PPP1R9B,CCDC110,SLMAP,ULBP2,NRCAM,B3GNT7, ATIC,LPAR5,SPATS2,NAB2,MRPS14,MMP11,CELF4,SHISA5,SAP30L,CX3CL1, KCNJ2,AOX1,CLCNKB,SMAD4,SETBP1,NINJ1,CYP20A1,GJB3,TGFB1,NCKIPSD, GHR,C19orf26,AP2A1,C8orf48,NUS1,ACSM2B,SPEF2,LAMB3,POLR3F,INF2,PIGN, AGRN,JAG2,C1GALT1,PKDREJ,HMGCR,FJX1,USP54,LZTS2,PKN3,POLI,ACACB, SHANK2,ACBD7,FN1,RHBDD2,TOMM34,TMEM19,AWAT2,XKRX,PLEKHG5,DZIP3, S100A11,THRSP,GABRD,TMEM220,FXYD5,FNDC4,PLEKHG4B,MPL,PAXIP1, IQGAP3,OCIAD2,RINL,CCND3,NOLC1
SP2	−4.87E-01	down	56	RC3H1,RAPGEF5,CDH24,BMP8A,DGKI,FAM63A,XPR1,SMAGP,PEA15,SREBF1, SNAPC2,CMTM3,SLC9A5,GATAD2A,RUNX1,TFPI,MDC1,IRF5,PPP1R13B,PRKCQ, KIAA1328,ALDH3B1,C11orf49,TNS1,ZFP36L2,LOC143666,HSDL1,ENTPD8,CITED2, IQCG,PDE4C,ICAM1,MGAT4B,TUSC3,GJA4,KLHDC8A,ISCA1,CDC14B,UBE2QL1, CHST2,SETDB2,NPC2,MTSS1L,CCND3,ACACB,MAT2A,DAB2IP,LASP1,SEZ6L2, CDH3,AP1S1,JAG2,CDH6,SYPL1,BANF2,CALCOCO2
FOXO3	−4.07E-01	down	56	SHROOM4,RTTN,SYTL3,LRRCC1,SMARCA4,CORO2A,PVRL4,UBE2I,MATN2, FAM19A5,NACC1,CDC14B,CCDC126,CYTH3,HCG18,TK1,GPR98,WWOX,WDR78, ARAP1,SLC6A1,PALM2,PTP4A3,NMNAT3,PIK3C2G,CAPN7,CDH13,GLRB,SPATS2, ISYNA1,GPR125,IPCEF1,TMEM117,SNX24,PDE9A,CCDC85A,SH2D4A,SLC35C1, SLC4A4,TTF1,KIAA1467,SFXN3,ZFYVE19,SYNE1,FUT1,DNAJC27,CEP68,SERINC2, TPD52L1,CHST2,GABRB2,FGFR2,ZNF22,PRTG,IGFBPL1,TPH2
EGR1	−1.87E + 00	down	49	

Table 5 (continued)

Transcription factor	logFC	Up/down	Count	Genes
				ULBP2,IER5,SLC4A4,FLII,ATXN7L1,STARD13,SYNE1,SLC9A5,MIA3,PCLO,STRN4, S100A16,C4orf46,MLLT1,FLJ23867,CDC14B,MTMR4,RRN3P3,CDH3,FAM126B, PDLIM3,FNDC4,TNS3,GALNT7,SNX24,CYB5D1,NINJ1,DLGAP4,NRCAM,ABR, BAIAP3,ANKRD27,SNAPC2,SPTBN2,ENDOD1,ME3,TBC1D2,LRP4,SH3RF1, EPHA2,ECE1,TNFRSF12A,SREBF1,LOC642852,MDK,TRAM2,TGFB1,ITGA3, ARHGAP6
ZEB1	−7.72E-01	down	45	EPHA2,RHOBTB3,MLLT1,CLCNKB,MTMR4,PIK3C2G,IRS1,PQLC2,CNOT4, GPRC5B,ZHX3,LRRK2,CTSH,KCNG4,CNTLN,RTKN,MRPS14,MYLK4,TSTA3, TUBD1,SHISA5,SSH3,INF2,CYP7B1,TIMP1,NPDC1,ME3,PCSK6,PRAF2,TBL1X, BCAR1,TMEM170B,LIMK1,PCGF5,SYTL1,SPEF2,DUSP13,NCS1,ATP2C2,DPP4, KIF6,MAP3K11,HCG18,ZFPM2,GHR
RREB1	−5.90E-01	down	30	HBB,TLE1,RASSF9,C11orf74,CARD10,CDH13,PTP4A3,ABR,ETV5,TIMP1,LPAR5,IQGAP3, EMILIN2,MAMLD1,NEGR1,SUV420H1,E2F1,SLC22A18,SYPL1,RHOC,PVRL4, TRIM41,ST8SIA4,KRT15,NCS1,FHOD1,PDGFA,HDAC4,DIRAS2,SMARCA2
EGR2	−2.48E + 00	down	26	SHANK2,FAM178B,AAK1,PLXNA3,CSNK1G1,MAP4K4,MAT2A,PAX6,TIAM1, CYP20A1,KIF6,DIRAS2,LOXL2,GSS,ARHGAP6,KIT,CDC42EP1,PRTG,SMARCA4, ELMO1,INF2,SCEL,ECE1,SAMD1,ABCC3,SNX24
SRF	−8.97E-01	down	23	ABR,EML6,GLI3,STRN4,SPATS2,GTF2E1,PROS1,SETBP1,SH2D4A,DNAH6, ARHGDIB,RYR2,DGKI,NEGR1,TBC1D4,PC,EML5,ABCC3,CNTLN,PTP4A3, SERPINH1,XPR1,TBL1X

Discussion

OS is a common bone cancer featured with aggressive tumors, metastatic and relapsing diseases [10]. However, metastases, chemoresistance and serious side effects still the main reasons for the failure of OS treatment [11]. Therefore, identifying molecular targets of the OS will be important to the development of strategies to improve patient outcomes [12, 13].

In this study, a set of 932 DEGs (475 up-regulated and 457 down-regulated) in OS compared with normal tissues were identified by integrated analysis of 9 microarray data of OS. Functional annotation showed that these DEGs were significantly involved in the p53 signaling pathway, Jak-STAT signaling pathway and Wnt signaling pathway.

To further obtain more information about TFs involved in OS, differentially expressed TFs and their target genes were identified by TRANSFAC. Then, the regulatory networks were constructed including 819 TF-target interactions including 46 TFs and 509 DEGs in OS. From the regulatory networks, we identified top 10 TFs with down-regulated expression, which covered the most downstream DEGs, including ZNF354C, NFIC, NFATC2, SP2, FOXO3, EGR1, ZEB1, RREB1, EGR2 and SRF. Validated expression levels of ZNF354C, NFIC, EGR1, SRF and EGR2 in GEO database were consistent with the bio-informatics result.

ZNF354C is a transcriptional repressor and is crucial to the development of osteoarthritis [14, 15]. Thus it can be seen that ZNF354C play roles in bone development. In this study, the expression of ZNF354C was down-regulated. This suggested that ZNF354C may function as a transcriptional repressor in the process of the OS.

NFIC belongs to NFI family, which plays roles in viral DNA replication, regulation of gene transcription, cell proliferation and development and it had been showed to be involved in OS progression via various biological processes and pathways [16]. Consistent with previous reports, we found NFIC indeed involved in the pathology of the OS and its diagnostic value was evaluated by ROC curve (AUC = 0.920, Specificity = 0.821, Sensitivity = 1.000).

NFATC2 is crucial to skeletal muscle growth [17]. It is reported that NFATC2-deficient mice develop osteoarthritis, osteomyelosclerosis and osteomyelofibrosis [18, 19]. In this study, we found the role of NFATC2 in the development of OS, which provided a new field in the treatment of the OS.

SP2 is a member of the SP family of transcription factors. It is found in several tumor cell lines and de-regulation of SP2

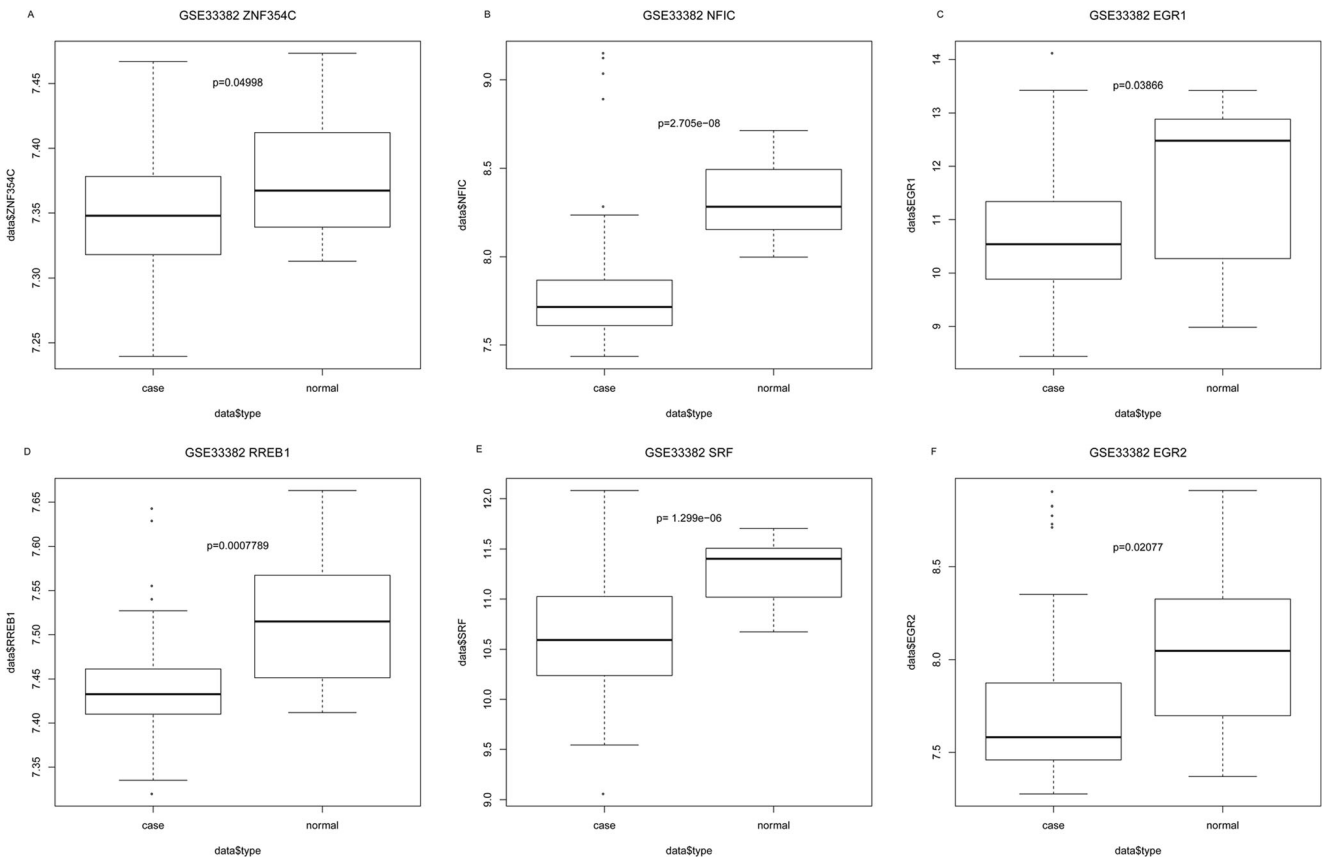

Fig. 5 The validation of the expression levels of selected genes in the OS based on GSE 16088 database. The x-axis shows the disease and control groups and y-axis shows expression reads counts. Disease group and control group indicated OS tissues and adjacent non-tumor tissues. **a**: ZNF354C, **b**: NFIC, **c**: EGR1, **d**: RREB1, **e**: SRF, **f**: EGR2

has also been associated with tumorigenesis [20, 21]. Herein, we found the expression of SP2 was down-regulated that was related to the onset of the OS.

FOXO3 belongs to FOXO family, which plays an indispensable role in maintaining skeletal homeostasis [22, 23]. An altered expression of FOXO3 has been involved in the severity of rheumatoid arthritis [24, 25]. In this study, the expression of FOXO3 was down-regulated and may involve in the process of the OS.

EGR1 functions as either a growth promoter or a tumor suppressor. It is demonstrated that expression of EGR1 decreased in OS cell lines and patient' biopsy specimens with reducing the invasion of OS [26]. In this study, we also found decreased expression of EGR1 in OS, which further demonstrated the role of EGR1 in integrating the mechanisms of OS.

ZEB1 has been considered as an important player in cancer process [27]. It is found that the defection of ZEB1 can inhibit the number of bone metastasis in mouse model [27]. Shen et al. also demonstrate that knockdown of ZEB1 will decrease the migration ability of OS cell [28]. In this study, we found the expression of ZEB1 was down-regulated which may influence the bone metastasis in OS.

RREB1 is found to function as an inducer or repressor of gene expression [29]. It binds the *p53* promoter and transactivates p53 expression on DNA damage in OS cells [30]. In this study, we found decreased expression of RREB1, which may play a significant role in DNA protection in OS.

EGR2 is a key regulatory factor in cell proliferation and cycle [31, 32]. It is reported that EGR2 can function in mediating the survival in any cell type [33]. It is worth mentioning that EGR2 has been involved in skeletal development [34]. In our study, we discovered the role of EGR2 in OS, which provided a new therapeutic method of OS.

SRF is shown to regulate the expression of genes with various biological processes, including cell proliferation, differentiation, survival, apoptosis and migration [35]. SRF is also critical in maintaining normal function of skeletal muscle and modulating osteoblast mineralization and bone homeostasis [36, 37]. Herein, we found the additional role of SRF in the process of the OS.

According to the KEGG analysis, the p53 signaling pathway, Jak-STAT signaling pathway and Wnt signaling pathway was three significantly pathways of DEGs. Chandar et al.

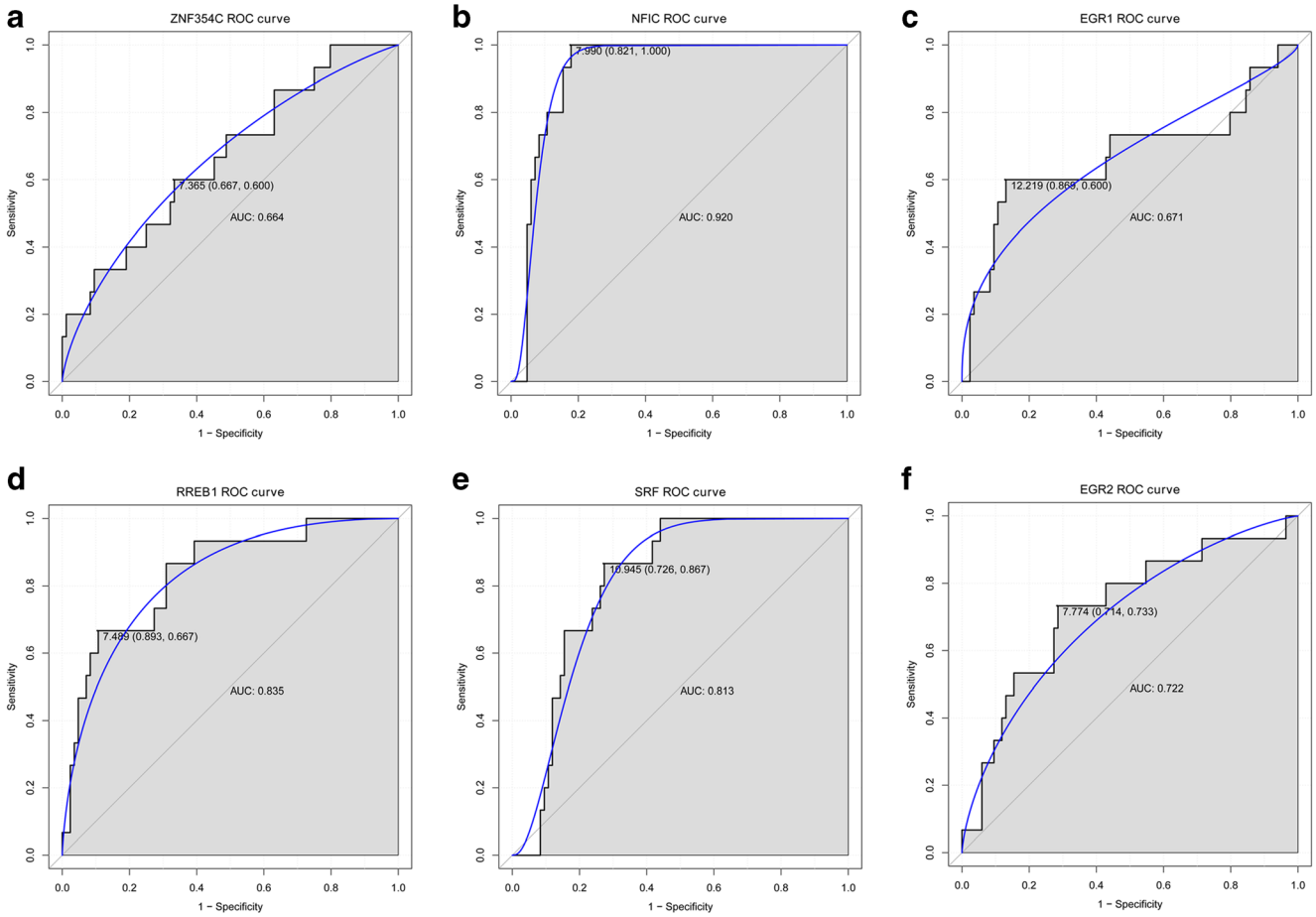

Fig. 6 The ROC curves were used to show the diagnostic ability of these selected genes with sensitivity and specificity. The x-axis shows 1-specificity and y-axis shows sensitivity: **a**: ZNF354C, **b**: NFIC, **c**: EGR1, **d**: RREB1, **e**: SRF, **f**: EGR2

proposed that the interaction between p53 and β-catenin pathway played an important role in osteoblast differentiation and bone tissue homeostasis [38]. It is noted that loss of p53 gene functions and mutation has been found in OS [39]. Moreover, it is confirmed that p53 is a negative prognostic marker of OS [40]. Activation of JAK2/STAT3 signalling pathway influences the expression of numerous proteins in cell cycle regulation and apoptosis. The signaling cascade has been known to contribute to tumorigenesis. It is reported that the inactivation of STAT3 by inhibiting JAK2 will reduce the proliferation, migration and invasion of OS cells [41]. The Wnt pathway is very important in many human cancers, particularly in somatic carcinoma [42]. Previous studies have reported that active Wnt signaling is associated with osteosarcoma development [43, 44]. Moreover, dysfunction of Wnt signaling will decrease metastatic capacity of OS cells [45, 46]. In addition, it is suggested that the derepression of Wnt signaling in osteoblasts may increase susceptibility to OS [44]. Our study further demonstrated the roles of p53 signaling pathway, Jak-STAT signaling pathway and Wnt signaling pathway in the development of OS.

Conclusions

In a word, our study provided available information to deeply understand the molecular mechanism in OS tumorigenesis. These findings revealed several important differentially expressed genes under the regulation of TFs and signaling pathways may provide an important clinical significance in OS.

Compliance with Ethical Standards

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

Research Involving Human Participants and/or Animals Not applicable.

Informed Consent Not applicable.

References

1. Koyani CN, Kitz K, Rossmann C, Bernhart E, Huber E, Trummer C, Windischhofer W, Sattler W, Malle E (2016) Activation of the MAPK/Akt/Nrf2-Egr1/HO-1-GCLc axis protects MG-63 osteosarcoma cells against 15d-PGJ2-mediated cell death. Biochem Pharmacol 104:29–41. https://doi.org/10.1016/j.bcp.2016.01.011
2. Mirabello L, Troisi RJ, Savage SA (2009) Osteosarcoma incidence and survival rates from 1973 to 2004: data from the surveillance, epidemiology, and end results program. Cancer 115(7):1531–1543. https://doi.org/10.1002/cncr.24121
3. Ottaviani G, Jaffe N (2009) The epidemiology of osteosarcoma. Cancer Treat Res 152:3–13. https://doi.org/10.1007/978-1-4419-0284-9_1
4. Longhi A, Errani C, De Paolis M, Mercuri M, Bacci G (2006) Primary bone osteosarcoma in the pediatric age: state of the art. Cancer Treat Rev 32(6):423–436. https://doi.org/10.1016/j.ctrv.2006.05.005
5. Gorlick R (2009) Current concepts on the molecular biology of osteosarcoma. Cancer Treat Res 152:467–478. https://doi.org/10.1007/978-1-4419-0284-9_27
6. Mirabello L, Koster R, Moriarity BS, Spector LG, Meltzer PS, Gary J, Machiela MJ, Pankratz N, Panagiotou OA, Largaespada D, Wang Z, Gastier-Foster JM, Gorlick R, Khanna C, de Toledo SR, Petrilli AS, Patino-Garcia A, Sierrasesumaga L, Lecanda F, Andrulis IL, Wunder JS, Gokgoz N, Serra M, Hattinger C, Picci P, Scotlandi K, Flanagan AM, Tirabosco R, Amary MF, Halai D, Ballinger ML, Thomas DM, Davis S, Barkauskas DA, Marina N, Helman L, Otto GM, Becklin KL, Wolf NK, Weg MT, Tucker M, Wacholder S, Fraumeni JF Jr, Caporaso NE, Boland JF, Hicks BD, Vogt A, Burdett L, Yeager M, Hoover RN, Chanock SJ, Savage SA (2015) A genome-wide scan identifies variants in NFIB associated with metastasis in patients with osteosarcoma. Cancer Discov 5(9):920–931. https://doi.org/10.1158/2159-8290.cd-15-0125
7. Johnson DS, Mortazavi A, Myers RM, Wold B (2007) Genome-wide mapping of in vivo protein-DNA interactions. Science (New York, NY) 316(5830):1497–1502. https://doi.org/10.1126/science.1141319
8. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A (2013) NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 41(Database issue):D991–D995. https://doi.org/10.1093/nar/gks1193
9. Knuppel R, Dietze P, Lehnberg W, Frech K, Wingender E (1994) TRANSFAC retrieval program: a network model database of eukaryotic transcription regulating sequences and proteins. J Comput Biol 1(3):191–198
10. Gibbs CP Jr, Weber K, Scarborough MT (2002) Malignant bone tumors. Instr Course Lect 51:413–428
11. Scotlandi K, Picci P, Kovar H (2009) Targeted therapies in bone sarcomas. Curr Cancer Drug Targets 9(7):843–853
12. Ragland BD, Bell WC, Lopez RR, Siegal GP (2002) Cytogenetics and molecular biology of osteosarcoma. Lab Investig 82(4):365–373
13. Clark JC, Dass CR, Choong PF (2008) A review of clinical and molecular prognostic factors in osteosarcoma. J Cancer Res Clin Oncol 134(3):281–297. https://doi.org/10.1007/s00432-007-0330-x
14. Meng F, Zhu L, Huang W, Irwin DM, Zhang S (2016) Bats: body mass index, forearm mass index, blood glucose levels and SLC2A2 genes for diabetes. Sci Rep 6:29960. https://doi.org/10.1038/srep29960
15. Fei Q, Lin J, Meng H, Wang B, Yang Y, Wang Q, Su N, Li J, Li D (2016) Identification of upstream regulators for synovial expression signature genes in osteoarthritis. Joint Bone Spine 83(5):545–551. https://doi.org/10.1016/j.jbspin.2015.09.001
16. Luo Y, Deng Z, Chen J (2013) Pivotal regulatory network and genes in osteosarcoma. Arch Med Sci 9(3):569–575. https://doi.org/10.5114/aoms.2012.30956
17. Horsley V, Friday BB, Matteson S, Kegley KM, Gephart J, Pavlath GK (2001) Regulation of the growth of multinucleated muscle cells by an NFATC2-dependent pathway. J Cell Biol 153(2):329–338
18. Wang J, Gardner BM, Lu Q, Rodova M, Woodbury BG, Yost JG, Roby KF, Pinson DM, Tawfik O, Anderson HC (2009) Transcription factor Nfat1 deficiency causes osteoarthritis through dysfunction of adult articular chondrocytes. J Pathol 219(2):163–172. https://doi.org/10.1002/path.2578
19. Bauer W, Rauner M, Haase M, Kujawski S, Arabanian LS, Habermann I, Hofbauer LC, Ehninger G, Kiani A (2011)

Osteomyelosclerosis, anemia and extramedullary hematopoiesis in mice lacking the transcription factor NFATc2. Haematologica 96(11):1580–1588. https://doi.org/10.3324/haematol.2011.042515
20. Kingsley C, Winoto A (1992) Cloning of GT box-binding proteins: a novel Sp1 multigene family regulating T-cell receptor gene expression. Mol Cell Biol 12(10):4251–4261
21. Kim TH, Chiera SL, Linder KE, Trempus CS, Smart RC, Horowitz JM (2010) Overexpression of transcription factor sp2 inhibits epidermal differentiation and increases susceptibility to wound- and carcinogen-induced tumorigenesis. Cancer Res 70(21):8507–8516. https://doi.org/10.1158/0008-5472.can-10-1213
22. Ambrogini E, Almeida M, Martin-Millan M, Paik JH, Depinho RA, Han L, Goellner J, Weinstein RS, Jilka RL, O'Brien CA, Manolagas SC (2010) FoxO-mediated defense against oxidative stress in osteoblasts is indispensable for skeletal homeostasis in mice. Cell Metab 11(2):136–146. https://doi.org/10.1016/j.cmet.2009.12.009
23. Almeida M (2011) Unraveling the role of FoxOs in bone–insights from mouse models. Bone 49(3):319–327. https://doi.org/10.1016/j.bone.2011.05.023
24. Turrel-Davin F, Tournadre A, Pachot A, Arnaud B, Cazalis MA, Mougin B, Miossec P (2010) FoxO3a involved in neutrophil and T cell survival is overexpressed in rheumatoid blood and synovial tissue. Ann Rheum Dis 69(4):755–760. https://doi.org/10.1136/ard.2009.109991
25. Lee JC, Espeli M, Anderson CA, Linterman MA, Pocock JM, Williams NJ, Roberts R, Viatte S, Fu B, Peshu N, Hien TT, Phu NH, Wesley E, Edwards C, Ahmad T, Mansfield JC, Gearry R, Dunstan S, Williams TN, Barton A, Vinuesa CG, Parkes M, Lyons PA, Smith KG (2013) Human SNP links differential outcomes in inflammatory and infectious disease to a FOXO3-regulated pathway. Cell 155(1):57–69. https://doi.org/10.1016/j.cell.2013.08.034
26. Matsunoshita Y, Ijiri K, Ishidou Y, Nagano S, Yamamoto T, Nagao H, Komiya S, Setoguchi T (2011) Suppression of osteosarcoma cell invasion by chemotherapy is mediated by urokinase plasminogen activator activity via up-regulation of EGR1. PLoS One 6(1):e16234. https://doi.org/10.1371/journal.pone.0016234
27. Liu Y, Zhang N, Wang Y, Xu M, Liu N, Pang X, Cao J, Ma N, Pang H, Liu L, Zhang H (2012) Zinc finger E-box binding homeobox 1 promotes invasion and bone metastasis of small cell lung cancer in vitro and in vivo. Cancer Sci 103(8):1420–1428. https://doi.org/10.1111/j.1349-7006.2012.02347.x
28. Shen A, Zhang Y, Yang H, Xu R, Huang G (2012) Overexpression of ZEB1 relates to metastasis and invasion in osteosarcoma. J Surg Oncol 105(8):830–834. https://doi.org/10.1002/jso.23012
29. Nitz MD, Harding MA, Smith SC, Thomas S, Theodorescu D (2011) RREB1 transcription factor splice variants in urologic cancer. Am J Pathol 179(1):477–486. https://doi.org/10.1016/j.ajpath.2011.03.038
30. Liu H, Hew HC, Lu ZG, Yamaguchi T, Miki Y, Yoshida K (2009) DNA damage signalling recruits RREB-1 to the p53 tumour suppressor promoter. Biochem J 422(3):543–551. https://doi.org/10.1042/bj20090342
31. Li X, Zhang Z, Yu M, Li L, Du G, Xiao W, Yang H (2013) Involvement of miR−20a in promoting gastric cancer progression by targeting early growth response 2 (EGR2). Int J Mol Sci 14(8):16226–16239. https://doi.org/10.3390/ijms140816226
32. Unoki M, Nakamura Y (2001) Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway. Oncogene 20(33):4457–4465. https://doi.org/10.1038/sj.onc.1204608
33. Bradley E, Ruan M, Oursler MJ (2008) Novel pro-survival functions of the Kruppel-like transcription factor Egr2 in promotion of macrophage colony-stimulating factor-mediated osteoclast survival downstream of the MEK/ERK pathway. J Biol Chem 283(12):8055–8064
34. Levi G, Topilko P, Schneider-Maunoury S, Lasagna M, Mantero S, Cancedda R, Charnay P (1996) Defective bone formation in Krox-20 mutant mice. Development 122(1):113–120
35. Miano JM (2003) Serum response factor: toggling between disparate programs of gene expression. J Mol Cell Cardiol 35(6):577–593
36. Li S, Czubryt MP, McAnally J, Bassel-Duby R, Richardson JA, Wiebel FF, Nordheim A, Olson EN (2005) Requirement for serum response factor for skeletal muscle growth and maturation revealed by tissue-specific gene deletion in mice. Proc Natl Acad Sci U S A 102(4):1082–1087. https://doi.org/10.1073/pnas.0409103102
37. Chen J, Yuan K, Mao X, Miano JM, Wu H, Chen Y (2012) Serum response factor regulates bone formation via IGF-1 and Runx2 signals. J Bone Miner Res Off J Am Soc Bone Miner Res 27(8):1659–1668. https://doi.org/10.1002/jbmr.1607
38. Chandar N, Saluja R, Lamar PC, Kolman K, Prozialeck WC (2005) P53 and beta-catenin activity during estrogen treatment of osteoblasts. Cancer Cell Int 5(24). https://doi.org/10.1186/1475-2867-5-24
39. Mendoza S, Konishi T, Dernell WS, Withrow SJ, Miller CW (1998) Status of the p53, Rb and MDM2 genes in canine osteosarcoma. Anticancer Res 18(6a):4449–4453
40. Bongiovanni L, Mazzocchetti F, Malatesta D, Romanucci M, Ciccarelli A, Buracco P, De Maria R, Palmieri C, Martano M, Morello E, Maniscalco L, Della Salda L (2012) Immunohistochemical investigation of cell cycle and apoptosis regulators (survivin, beta-catenin, p53, caspase 3) in canine appendicular osteosarcoma. BMC Vet Res 8:78. https://doi.org/10.1186/1746-6148-8-78
41. Angulo P, Kaushik G, Subramaniam D, Dandawate P, Neville K, Chastain K, Anant S (2017) Natural compounds targeting major cell signaling pathways: a novel paradigm for osteosarcoma therapy. J Hematol Oncol 10(1):10. https://doi.org/10.1186/s13045-016-0373-z
42. Thomas DM (2010) Wnts, bone and cancer. J Pathol 220(1):1–4. https://doi.org/10.1002/path.2635
43. Iwaya K, Ogawa H, Kuroda M, Izumi M, Ishida T, Mukai K (2003) Cytoplasmic and/or nuclear staining of beta-catenin is associated with lung metastasis. Clin Exp Metastasis 20(6):525–529
44. Kansara M, Tsang M, Kodjabachian L, Sims NA, Trivett MK, Ehrich M, Dobrovic A, Slavin J, Choong PF, Simmons PJ, Dawid IB, Thomas DM (2009) Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. J Clin Invest 119(4):837–851. https://doi.org/10.1172/jci37175
45. Hoang BH, Kubo T, Healey JH, Yang R, Nathan SS, Kolb EA, Mazza B, Meyers PA, Gorlick R (2004) Dickkopf 3 inhibits invasion and motility of Saos-2 osteosarcoma cells by modulating the Wnt-beta-catenin pathway. Cancer Res 64(8):2734–2739
46. Guo Y, Zi X, Koontz Z, Kim A, Xie J, Gorlick R, Holcombe RF, Hoang BH (2007) Blocking Wnt/LRP5 signaling by a soluble receptor modulates the epithelial to mesenchymal transition and suppresses met and metalloproteinases in osteosarcoma Saos-2 cells. J Orthop Res 25(7):964–971. https://doi.org/10.1002/jor.20356