Slow skeletal muscle troponin T, titin and myosin light chain 3 are candidate prognostic biomarkers for Ewing's sarcoma

YAJUN DENG[1,2*], QIQI XIE[1,2*], GUANGZHI ZHANG[1,2*], SHAOPING LI[1,2], ZUOLONG WU[1,2], ZHANJUN MA[1,2], XUEGANG HE[1,2], YICHENG GAO[1,2], YONGGANG WANG[1], XUEWEN KANG[1,2] and JING WANG[1,2]

[1]Department of Orthopedics; [2]Key Laboratory of Orthopedic Disease of Gansu Province, Lanzhou University Second Hospital, Lanzhou, Gansu 730030, P.R. China

Received March 16, 2019; Accepted September 17, 2019

DOI: 10.3892/ol.2019.11044

Abstract. Ewing's sarcoma (ES) is a common malignant bone tumor in children and adolescents. Although great efforts have been made to understand the pathogenesis and development of ES, the underlying molecular mechanism remains unclear. The present study aimed to identify new key genes as potential biomarkers for the diagnosis, targeted therapy or prognosis of ES. mRNA expression profile chip data sets GSE17674, GSE17679 and GSE45544 were downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) were screened using the R software limma package, and functional and pathway enrichment analyses were performed using the enrichplot package and GSEA software. The NetworkAnalyst online tool, as well as Cytoscape and its plug-ins cytoHubba and NetworkAnalyzer, were used to construct a protein-protein interaction network (PPI) and conduct module analysis to screen key (hub) genes. LABSO COX regression and overall survival (OS) analysis of the Hub genes were performed. A total of 211 DEGs were obtained by integrating and analyzing the three data sets. The functions and pathways of the DEGs were mainly associated with the regulation of small-molecule metabolic processes, cofactor-binding, amino acid, proteasome and ribosome biosynthesis in eukaryotes, as well as the Rac1, cell cycle and P53 signaling pathways. A total of one important module and 20 hub genes were screened from the PPI network using the Maximum Correlation Criteria algorithm of cytoHubba. LASSO COX regression results revealed that titin (*TTN*), fast skeletal muscle troponin T, skeletal muscle actin α-actin, nebulin, troponin C type 2 (fast), myosin light-chain 3 (*MYL3*), slow skeletal muscle troponin T (*TNNT1*), myosin-binding protein C1 slow-type, tropomyosin 3 and myosin heavy-chain 7 were associated with prognosis in patients with ES. The Kaplan-Meier curves demonstrated that high mRNA expression levels of *TNNT1* (P<0.001), *TTN* (P=0.049), titin-cap (P=0.04), tropomodulin 1 (P=0.011), troponin I2 fast skeletal type (P=0.021) and *MYL3* (P=0.017) were associated with poor OS in patients with ES. In conclusion, the DEGs identified in the present study may be key genes in the pathogenesis of ES, three of which, namely *TNNT1*, *TTN* and *MYL3*, may be potential prognostic biomarkers for ES.

Introduction

Ewing's sarcoma (ES) is the second most common primary malignant bone tumor after osteosarcoma, and it occurs in children and adolescents (1,2). ES is extremely malignant, with a short course of disease, rapid recurrence and high transfer rate (3). With the continuous improvements in ES treatment, the current 5-year survival rate is between 65 and 75% (4). However, owing to the lack of effective diagnostic methods in the early stages of the disease, ~25% of patients with ES still experience distant metastasis, which results in poor prognosis (5,6). Therefore, clarifying the precise molecular mechanisms involved in the development of ES is essential to develop effective diagnostic and therapeutic strategies.

Abnormal expression and mutations of genes are involved in the development and progression of ES. A previous study has reported that nuclear phosphoprotein (*NPM*) promotes proliferation and invasion of ES cells, and elevated *NPM* expression may be associated with poor prognosis of ES (7). Insulin-like growth factor 1 (*IGF1*) and its receptor (IGF1-R) serve a key role in the progression of ES by interfering with the IGF1R pathway in ES cells and subsequently inhibiting cell proliferation, promoting apoptosis and reducing invasion and metastasis (8,9). KIT proto-oncogene receptor tyrosine kinase is a tyrosine kinase receptor that is significantly expressed in ES associated with the proliferative, invasive and metastatic

Correspondence to: Professor Xuewen Kang or Professor Jing Wang, Department of Orthopedics, Lanzhou University Second Hospital, 82 Cuiyingmen Street, Lanzhou, Gansu 730030, P.R. China
E-mail: ery_kangxw@lzu.edu.cn
E-mail: wang_jing@lzu.edu.cn

*Contributed equally

Abbreviations: ES, Ewing's sarcoma; GEO, Gene Expression Omnibus; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CC, cellular component; MF, molecular function; BP biological process

Key words: Ewing's sarcoma, differentially expressed genes, bioinformatics, prognosis, biomarkers

ability of ES cells (10). Stromal antigen 2 mutation occurs in 20% of ES cases and is associated with distant metastasis, although whether it may be considered a prognostic marker for ES remains controversial (11). Other molecular genetic alterations associated with ES include abnormal expression of platelet-derived growth factor receptor β (12,13) and mammalian target of rapamycin (14), as well as *CDKN2A* and *TP53* mutations (15,16). A better understanding of the molecular biology of ES may help identify novel early diagnostic biomarkers, potential therapeutic targets or prognostic indicators.

In recent decades, gene chip technology and bioinformatics have been widely used to screen genetic changes at the genomic level, which can identify the differentially expressed genes (DEGs) and functional pathways involved in ES carcinogenesis and progression. In this study, three mRNA expression profile chip data sets from Gene Expression Omnibus (GEO) (17) were downloaded and analyzed to obtain DEGs between ES and normal tissues. Functional and pathway enrichment analysis and protein-protein interaction (PPI) network analysis of DEGs were also performed. Finally, LASSO COX regression model and overall survival rate (OS) analysis of hub genes were performed. The results provided a useful framework for elucidating the pathogenesis of ES and its complex molecular biology and for identifying new key genes that may be used as prognostic biomarkers for ES.

Materials and methods

Microarray data. A total of three gene-expression data sets were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo) (17): GSE17674 (18), GSE17679 (18) and GSE45544 (19). The GSE17674 data set was based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array) and included 44 samples from patients with ES and 18 adjacent non-cancerous tissues from the patients. The GSE17679 data set was based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array) and included 88 patient samples and 18 adjacent non-cancerous tissues from the patients. The GSE45544 data set was based on the GPL6244 platform (Affymetrix Human Gene 1.0 ST Array) and included 14 patient samples and 22 normal tissues from healthy control subjects.

Data preprocessing and DEG screening. Raw data from the three data sets (CEL file) were read using the R language (version 3.1.2; http://r-project.org/) Affy package (20). The original CEL file was removed and the data were subjected to background correction, bootstrap correction, quality control and normalization processing. The R package sva (21) was used to perform batch effect removal on the three data sets. The data were converted into a probe expression matrix and analyzed by the R language limma package (22) to obtain DEGs. The DEGs were filtered by an adjusted P-value (adj.P-value) <0.05 and |log2 fold change (FC)|>1.

DEG function and pathway enrichment analysis. To analyze the functions of the DEGs, the R software enrichplot package (23) was used to perform GO and KEGG pathway enrichment analysis. P<0.05 was considered to indicate significant gene enrichment.

Gene Set Enrichment Analysis (GSEA) was performed using c2.cp.kegg.v6.0.symbols.gmt (http://software.broadinstitute.org/gsea/msigdb/download_file.jsp?filePath=/resources/msigdb/7.0/c2.cp.kegg.v7.0.symbols.gmt) (24) as a reference gene set. The GSEA software (version 6.3) is available on the GSEA website (http://software.broadinstitute.org/gsea/index.jsp). False Discovery Rate (FDR) <0.25 and P-value <0.05 were used as the cut-off criteria.

PPI network construction and hub gene selection. The NetworkAnalyst (http://www.networkanalyst.ca) online tool was used to build a PPI network of the DEGs with parameters set to a confidence score ≥900. The Cytoscape (version 3.6.1) software (25) was used to visualize the PPI network and identify the most important modules in the PPI network through the NetworkAnalyzer plugin (26). To extract valuable information from the important modules, the cytoHubba plugin (27) was used to identify the hub genes. The top 20 genes in Maximum Correlation Criteria (MCC) were selected by the cytoHubba plugin and sorted by enrichment fraction.

LASSO COX regression and overall survival (OS) analysis. The LASSO COX regression model and Kaplan-Meier OS analysis were used to screen genes with strong association with prognosis from the hub genes and assess their effects on survival in ES. LASSO coefficient profiles of the 10 ES-associated genes were analyzed, and a vertical line was drawn at the value selected by 10-fold cross-validation. OS analysis was performed by R-survival (28) and survminer (https://cran.r-project.org/web/packages/survminer/index.html) based on high- and low-expression levels of gene expression determined by BestSeparation (29), and data from genes with P<0.05 were retained for display.

Results

Identification of DEGs in ES. The R package sva (21) was used to perform batch effect removal on the three data sets. The principal component analysis (PCA) before and after batch effect removal is presented in Fig. 1A and B, respectively. The results demonstrated that the sample data set selected in the present study was of reliable quality. Following standardization of the chip results, 1,133, 1,290 and 768 DEGs between ES and healthy tissues were extracted from the GSE17674, GSE17679 and GSE45544 mRNA expression profile data sets, respectively. The overlap between the three data sets contained 211 DEGs, as presented in the Venn diagram (Fig. 1C).

Function and pathway enrichment analysis of DEGs. To determine the biological functions of the DEGs, GO and KEGG pathway enrichment analyses were performed using the R software enrichplot package (23). The results of GO analysis demonstrated that the BP changes of the DEGs were significantly enriched in 'response to peptides', 'regulation of body fluid levels', 'response to extracellular stimulus', 'regulation of small molecule metabolic process' and 'gland development' (Fig. 2A). Changes in MF were mainly enriched in 'cell adhesion molecule binding', 'cofactor binding', 'proximal promoter sequence-specific DNA binding', 'RNA polymerase II proximal promoter sequence-specific DNA binding' and

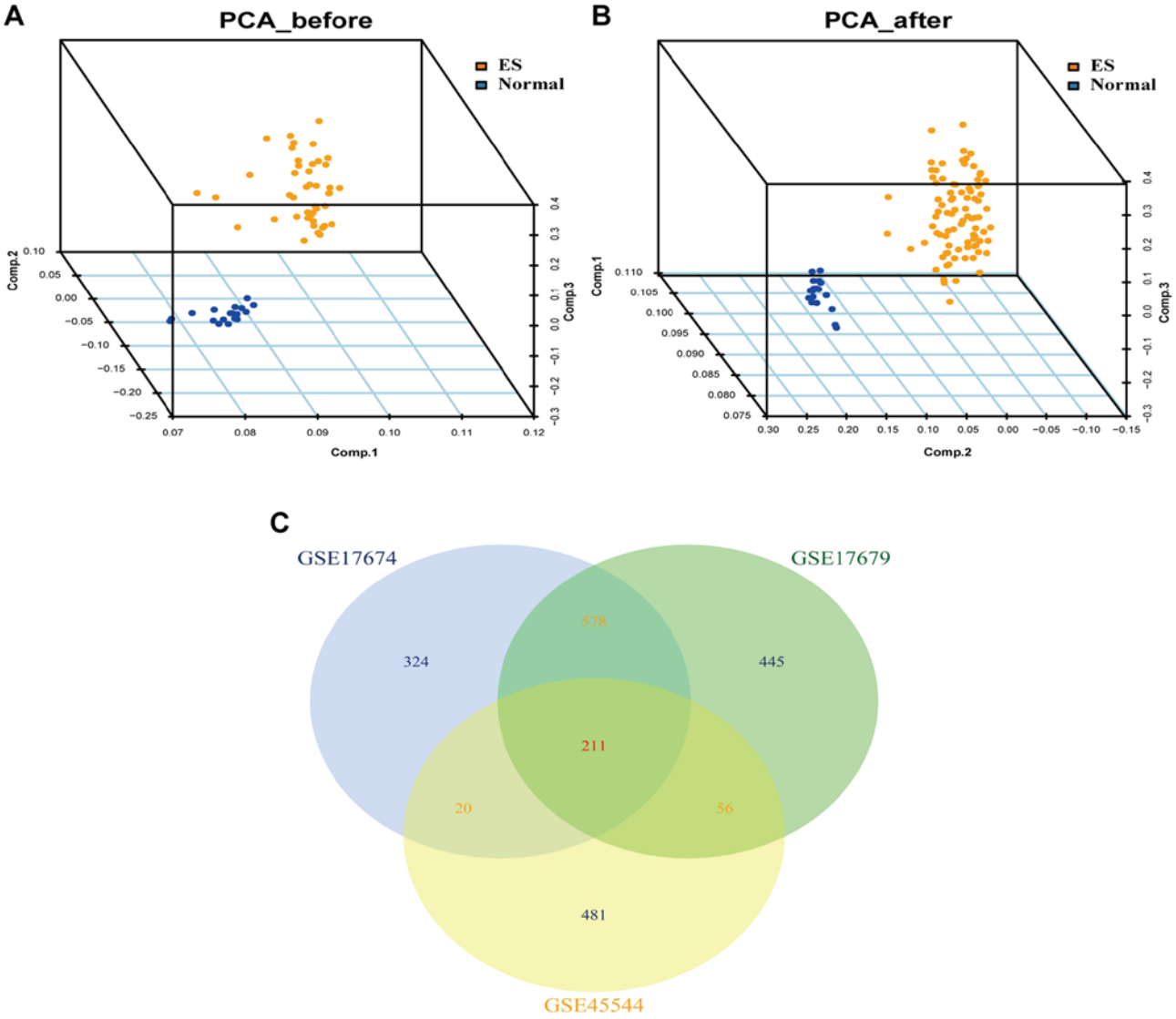

Figure 1. Sample pretreatment and Venn diagram. (A and B) PCA (A) before and (B) after batch effect removal. (C) Screening of DEGs. A total of 211 overlapping DEGs were identified in the three data sets. The DEGs satisfied the criteria adjusted P-value <0.05 and |log2 fold change|>1. PCA, principal component analysis; Comp, component; ES, Ewing's sarcoma.

'transcriptional activator activity-specific DNA-binding (Fig. 2A). The CC of DEGs varied mainly in 'neuronal cell body', 'cell-substrate junction', 'cell-substrate adherens junction', 'focal adhesion' and 'vesicle lumen' (Fig. 2A). The results of KEGG pathway enrichment analysis demonstrated that the DEGs were mainly enriched in 'amino acid biosynthesis', 'DNA replication', 'focal adhesion', 'proteasome' and 'ribosomal biogenesis in eukaryotes' (Fig. 2B). GSEA results revealed that the enriched functions and pathways mainly involved the p53 (P=0.004; NES=1.528), Rac1 (P=0.010; NES=1.544) and cell cycle (P=0.010; NES=1.542) pathways (Fig. 3).

PPI network construction and selection of hub genes. The NetworkAnalyst online tool was used to build a PPI network of DEGs to analyze PPIs (Fig. 4A). The module with the highest MCC score in this PPI network was identified using the NetworkAnalyzer plugin in the Cytoscape software (Fig. 4B). In addition, the cytoHubba plugin was used to select the top 20 genes in MCC as the hub genes: Titin (*TTN*), myosin light-chain 3 (*MYL3*), fast skeletal muscle troponin T, troponin C type 2 (fast), tropomyosin 3, *MYL2*, troponin C type 1 (slow), troponin I2 fast skeletal type (*TNNI2*), troponin I1 slow skeletal type, myosin-binding protein C fast type, myosin-binding protein C slow type, slow skeletal muscle troponin T (*TNNT1*), titin-cap (*TCAP*), nebulin, desmin, tropomodulin 1 (*TMOD1*), myosin heavy-chain 7 (*MYH7*), *MYH1*, *MYH2* and skeletal muscle α-actin (Fig. 4C). The functions of these hub genes are presented in Table I.

LASSO COX regression and OS analysis. LASSO COX regression and OS analysis of the 20 hub genes was performed. LASSO COX regression results demonstrated that 10 genes were associated with prognosis (Fig. 5), whereas Kaplan-Meier curves revealed that high mRNA expression levels of *TNNT1* (HR, 3.57; 95% CI, 2.06-6.18; P<0.001), *TTN* (HR, 1.82; 95% CI, 1.04-3.16; P=0.049), *TCAP* (HR, 1.74; 95% CI, 0.97-3.14; P=0.04), *TMOD1* (HR, 2.03; 95% CI, 1.18-3.50; P=0.011), *TNNI2* (HR, 1.94; 95% CI, 1.13-3.34; P=0.021) and *MYL3*

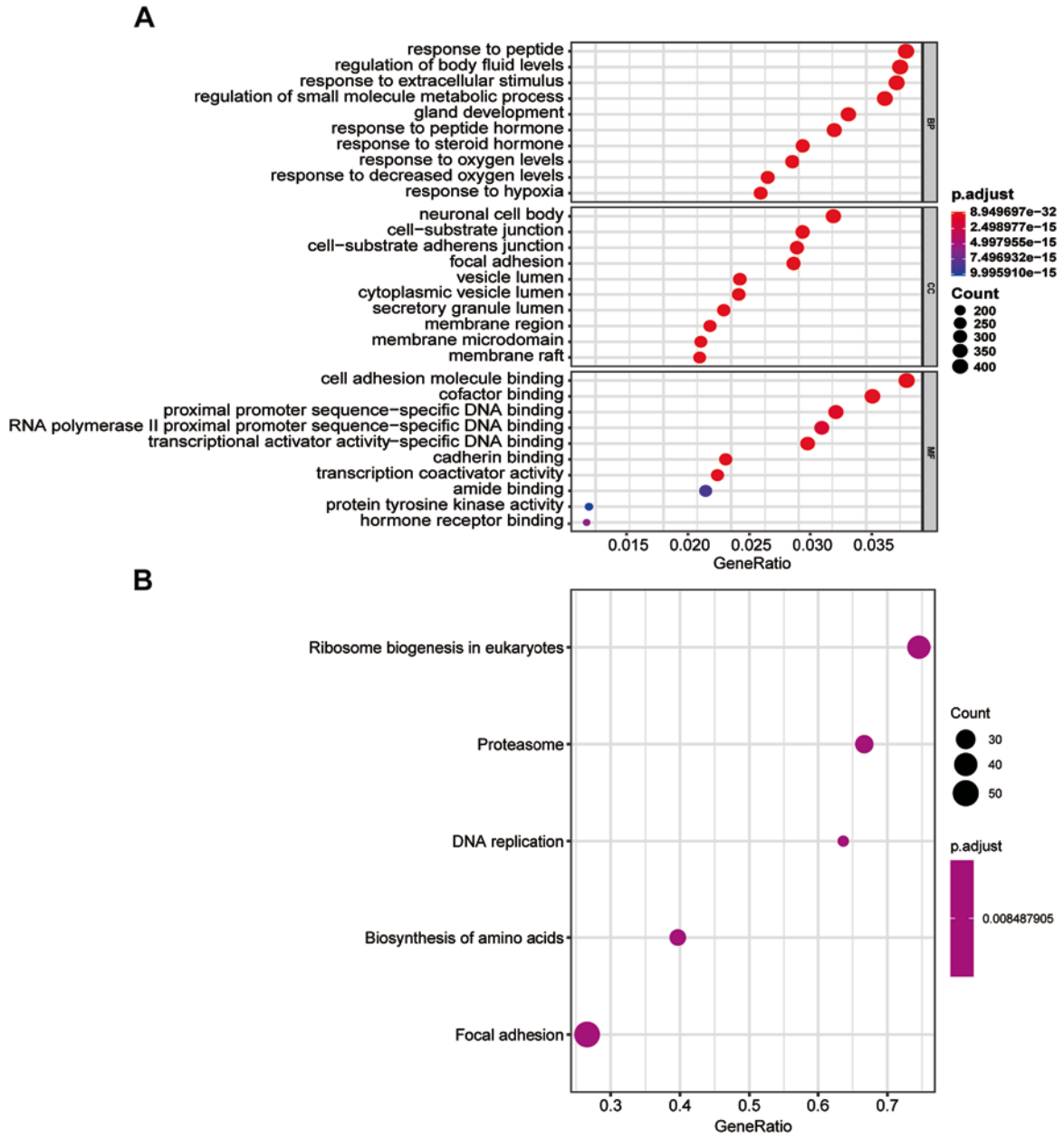

Figure 2. GO and KEGG pathway enrichment analysis. (A) GO analysis. The color of the dot represents the adjusted P-value: Red, low; blue, high. The size of the dots represents the number of DEGs. (B) KEGG pathway enrichment analysis. The color of the dots represents the adjusted P-value; the size of the dots represents the number of DEGs in the pathway. DEGs were mainly enriched in 'biosynthesis of amino acids', 'DNA replication', 'focal adhesion', 'proteasome' and 'ribosome biogenesis in eukaryotes'. GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; p.adjust, adjusted P-value; BP, biological process; CC, cellular component; MF, molecular function.

(HR, 2.02; 95% CI, 1.17-3.49; P=0.017) were associated with poor OS in patients with ES (Fig. 6). Based on these results, *TNNT1*, *TTN* and *MYL3* may be key players in the abnormal signaling pathway of ES and may serve as potential prognostic biomarkers for ES.

Discussion

ES is a cancer of the bones and muscles, but it also occurs in soft tissues (30,31). ES is the second most common primary malignant bone tumor in children and adolescents, with 2.9 people per million diagnosed with ES annually worldwide (32). The metastasis rate is ES was 20-25%, and the recurrence rate is 30-40% (33,34). Although multiple molecular studies on the pathogenesis of ES have been conducted (35-37), the underlying molecular mechanism is largely unknown. In addition, the prognosis of patients with ES remains uncertain. Therefore, there is an urgent need to identify novel potential biomarkers for early diagnosis, targeted therapy or prognostic evaluation of ES to improve the prognosis of patients with ES. Microarray analysis is a high-throughput technology that simultaneously detects the expression levels of thousands of genes. Microarray technology was used in the present study to explore genetic changes in ES, as numerous studies have demonstrated it to be an effective way to identify new biomarkers for other diseases (38,39). The present study aimed to enhance ES data through bioinformatics methods to improve the understanding of this disease.

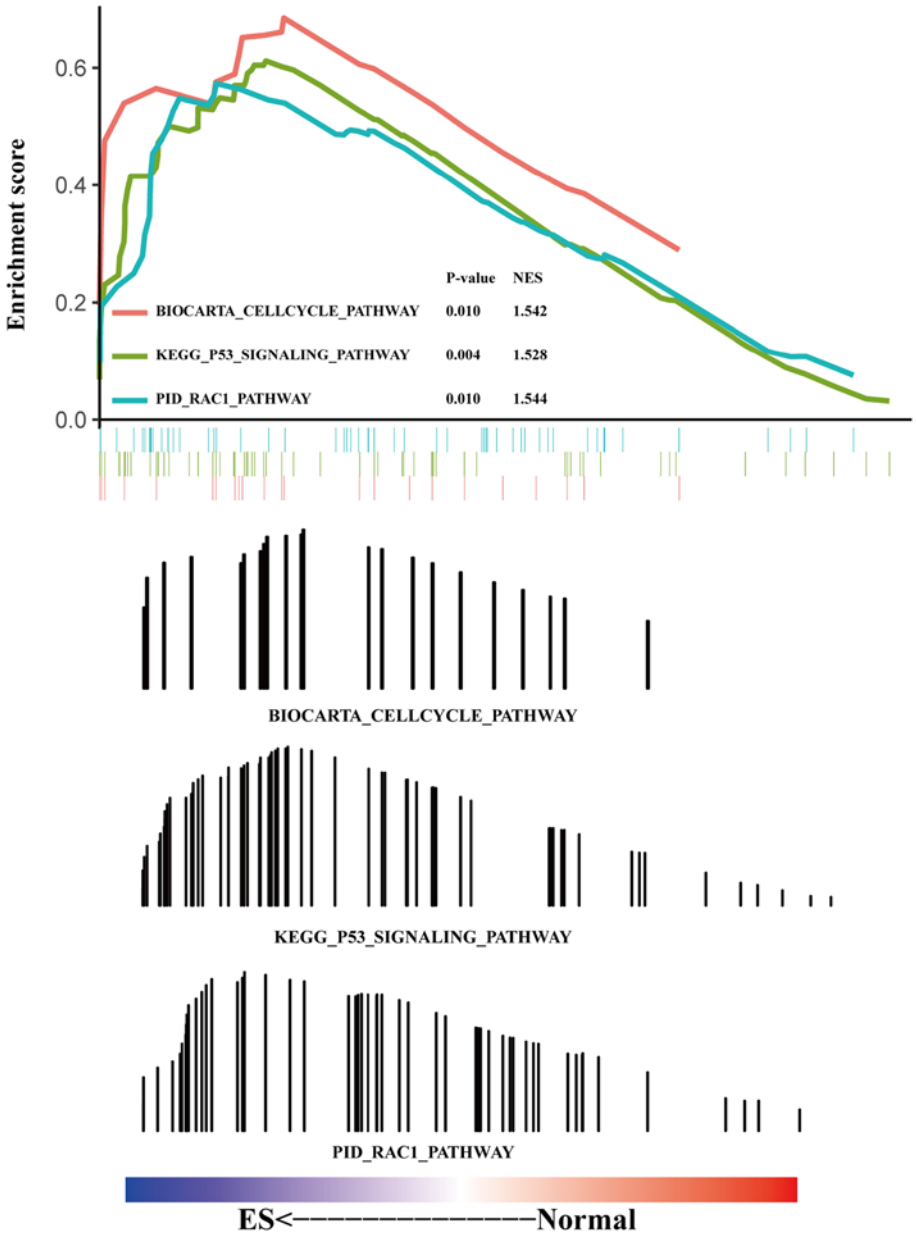

Figure 3. Gene Set Enrichment Analysis results. The red line represents the cell cycle pathway, the cyan line represents the P53 signaling pathway, and the blue line represents the Rac1 pathway. NES, Normalized Enrichment Score; KEGG, Kyoto Encyclopedia of Genes and Genomes; ES, Ewing's sarcoma; PID, the Pathway Interaction Database.

In the present study, three mRNA microarray data sets were downloaded from the GEO database, and an in-depth analysis was conducted using bioinformatics methods to obtain DEGs between ES and normal tissues. Prior to the analysis of the data sets, the inter-assay difference removal, background correction, bootstrap correction, quality control and standardization was performed to ensure that the data were valid for the next step of analysis. A total of 211 DEGs were identified in all three datasets between ES and normal tissues.

Since the general difference analysis (GO and KEGG) focuses on comparing gene expression differences between two groups and on genes that are significantly upregulated or downregulated, genes that are not significantly differentially expressed but serve important biological significance are easily omitted. This may lead to neglecting valuable information on the biological characteristics of certain genes, associations between gene regulatory networks, functions and significance of genes. The GSEA software does not need to specify a clear differential gene threshold; the algorithm performs enrichment analysis on all genes in the expression profile based on the overall trend of the actual data; mathematical statistics link the expression spectrum chip data with biological meaning to avoid missing important information (24). Therefore, GSEA, GO, and KEGG pathway enrichment analyses were performed in the present study; the results demonstrated that the DEGs were mainly involved in functions and pathways associated with ES development and progression, including the 'Rac1 pathway', 'cell cycle pathway', 'RNA metabolism' and 'P53 signaling pathway'. The Rac1 pathway is closely associated with the invasion and metastasis of ES; a previous study has demonstrated that Erb-B2 receptor tyrosine kinase 4 mediates Rac1 GTPase activation and enhances ES invasion and

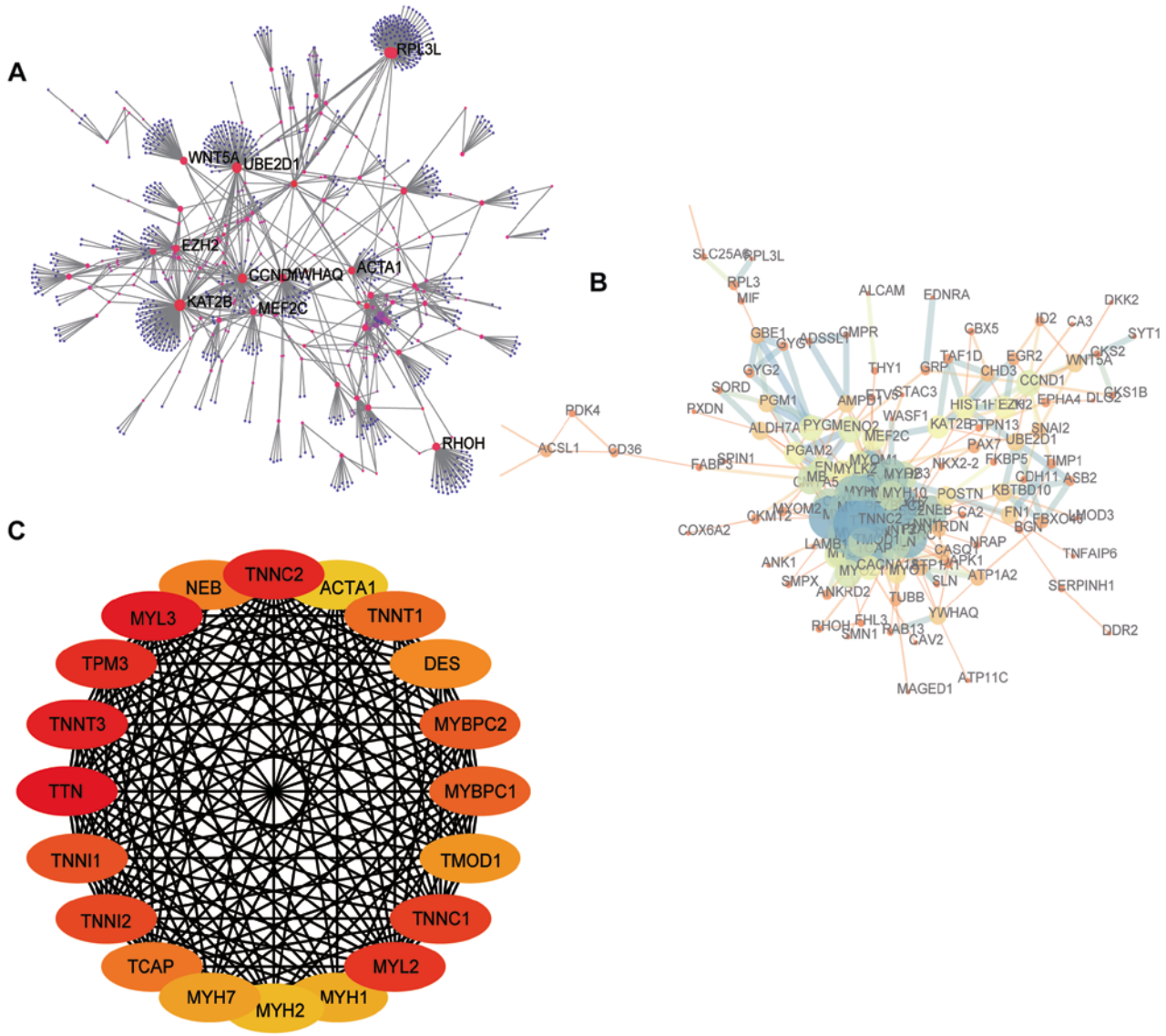

Figure 4. PPI network and hub genes. (A) A PPI network was constructed using the NetworkAnalyst online tool. (B) The NetworkAnalyzer plugin identified the most important modules in the PPI network. The node size represents the clustering coefficient and the proportion of genes in the network. The color of the node represents the degree (blue, high; yellow, medium; orange, low). The thickness of the connection represents the comprehensive score. (C) The cytoHubba plugin selected the top 20 genes in the Maximum Correlation Criteria as hub genes. PPI, protein-protein interaction.

metastasis *in vivo* by activating the PI3K-Akt cascade and focal adhesion kinase (40). The cell cycle pathway serves a key role in the malignant proliferation process in breast (41), endometrial (42), bladder (43) and gastric (44) cancer. A recent study reported that the cell cycle pathway is also involved in the ES proliferation process (45). In addition, the P53 and RNA metabolism signaling pathways serve an important role in the progression of ES (46,47). Collectively, these results are consistent with the present study.

A number of ES biomarkers in the literature match the DEGs identified in the present study. For example, Tong *et al* (48) analyzed and identified Fc fragment of immunoglobulin G receptor and transporter, olfactomedin 1, Cbp/P300-interacting transactivator with glu/asp, caveolin 1,3-ketodihydrosphingosine reductase, cadherin 3 (*CHD3*), growth arrest-specific 1, four and a half LIM domains 3 and thyroid hormone receptor interactor 6 as biomarkers of ES. Cheung *et al* (49) identified six-transmembrane epithelial antigen of prostate1, NK2 homeobox 2 and cyclin D1 as biomarkers of ES based on the gene expression array approach. Other studies have analyzed global genomic and transcriptomic expression to identify prognostic biomarkers for ES, and the results demonstrated that *CDH11* (50) and nucleophosmin 1 (51) were biomarkers of ES. All of the above genes were differentially regulated in the present study.

A PPI network of DEGs was constructed in the present study to detect interactions between the proteins coded by the DEGs, and one important module was extracted from the network. Subsequently, the top 20 genes in MCC were selected from the important module as the hub genes. To assess the effect of these 20 hub genes on survival in ES, survival analysis was performed using the LASSO COX regression and Kaplan-Meier curves. The results revealed that high mRNA expression levels of *TNNT1*, *TTN* and *MYL3* were significantly associated with poor OS in patients with ES, suggesting that these genes may serve an important role in the development of ES.

Table I. Functional roles of top 20 hub genes.

No.	Gene symbol	Gene name	Function
1	*TTN*	Titin	Mutation may serve a specific role in the development or progression of colorectal cancer
2	*MYL3*	Myosin light-chain 3	Mutations have been identified as a cause of mid-left ventricular chamber type hypertrophic cardiomyopathy
3	*TNNT3*	Fast skeletal muscle troponin T	Promotes the progression of breast cancer
4	*TNNC2*	Troponin C type 2 (fast)	Serves a key role in the regulation of muscle contraction and modulates the Ca^{2+}-activation characteristics of muscle fibers
5	*TPM3*	Tropomyosin 3	Potential biomarker for colorectal cancer
6	*MYL2*	Myosin light-chain 2	Associated with invasion, metastasis and poor prognosis of several cancers
7	*TNNC1*	Troponin C type 1 (slow)	Potential marker for predicting occult cervical lymphatic metastasis and prognosis of oral tongue carcinoma
8	*TNNI2*	Troponin I2, fast skeletal type	High expression in gastric tissue is a specific biomarker for peritoneal metastasis of gastric cancer
9	*TNNI1*	Troponin I1, slow skeletal type	Downregulation restrains proliferation of non-small-cell lung carcinoma xenografts
10	*MYBPC2*	Myosin-binding protein C, fast type	May modulate muscle contraction or serve a structural role
11	*MYBPC1*	Myosin-binding protein C, slow type	Missense and nonsense mutations have been directly linked with the development of severe and lethal forms of distal arthrogryposis myopathy and muscle tremors
12	*TNNT1*	Slow skeletal muscle troponin T	High expression is associated with cell proliferation and migration in a variety of cancers
13	*TCAP*	Titin-cap	Important for accurate diagnosis and prognosis of breast cancer subtypes
14	*NEB*	Nebulin	Mutation is involved in the development of osteosarcoma
15	*DES*	Desmin	Potential oncofetal diagnostic and prognostic biomarker in colorectal cancer
16	*TMOD1*	Tropomodulin 1	Involved in the development, invasion and metastasis of various tumors
17	*MYH7*	Myosin heavy-chain 7	Forms the basic contractile unit of skeletal muscle and myocardium
18	*MYH1*	Myosin heavy-chain 1	Involved in the development, invasion and metastasis of breast cancer
19	*MYH2*	Myosin heavy-chain 2	Potential driver of squamous cell lung cancer
20	*ACTA1*	Skeletal muscle α-actin gene	Involved in various types of cell movement and is commonly expressed in all eukaryotic cells

The protein encoded by *TNNT1* is a subunit of troponin, a regulatory complex located on the sarcomeric filament, which regulates the contraction of striated muscle in response to fluctuations in intracellular calcium concentration (52). Microinjections of tropomyosin into epithelial cells can induce rapid cell migration (53); *TNNT1* may contribute to the interaction between actin and tropomyosin, thus regulating cell migration and invasion (54). Rhabdomyosarcoma is a cancer associated with connective tissue, and the cause of this sarcoma is unclear (55). However, its malignant biological behavior may be associated with the expression of *TNNT1*, and *TNNT1* can be used as a biomarker for the diagnosis and prognosis of rhabdomyosarcoma (48). In addition, the expression of *TNNT1* is significantly increased in uterine sarcoma and closely associated with cell proliferation and migration (56,57). Highly expressed *TNNT1* has been demonstrated to be a prognostic biomarker for the development and poor prognosis of numerous types of cancer, such as breast and endometrial cancers. Shi *et al* (58) reported that the expression of *TNNT1* was significantly increased in breast cancer and was closely associated with clinical stage, tumor size and lymph node involvement. Further experiments demonstrated that *TNNT1* promoted the proliferation of breast cancer cells by promoting the G1/S transition (58). *TNNT1* is also upregulated in endometrial carcinoma, which indicates that *TNNT1* may be associated with the phenotype of invasive tumor cells, but

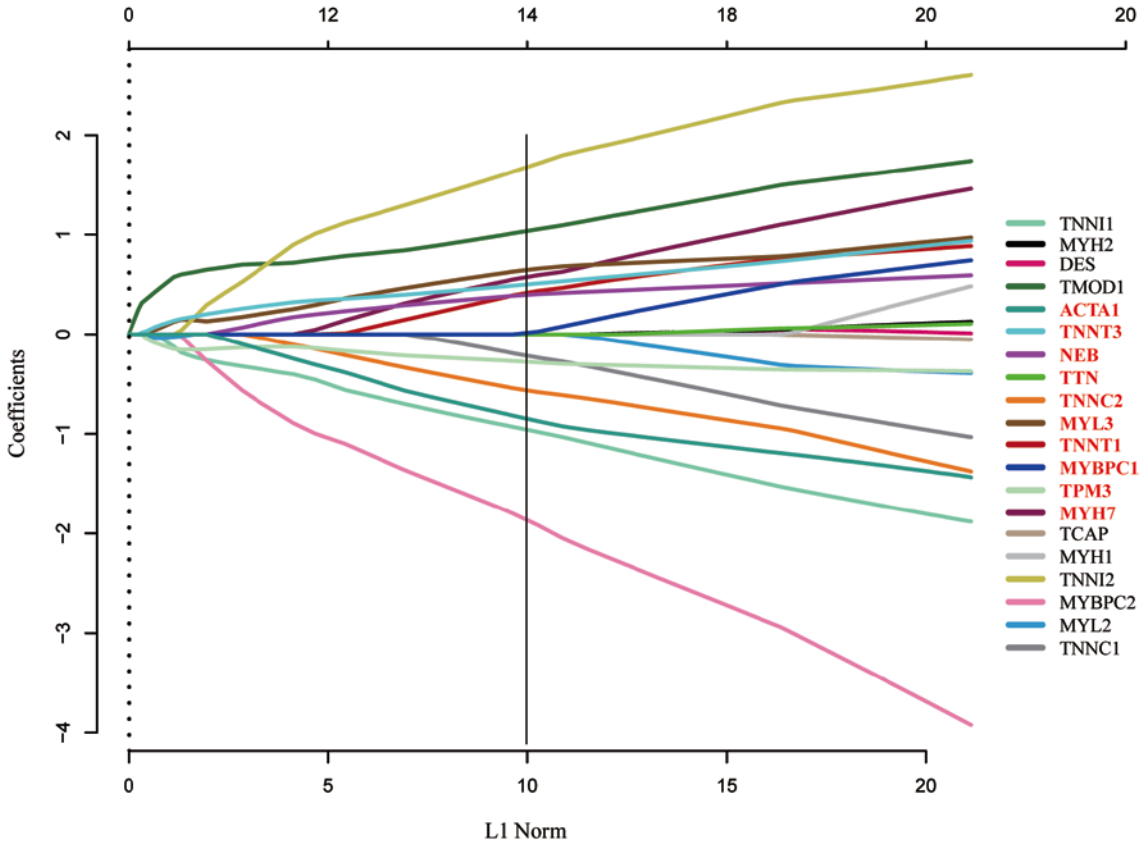

Figure 5. LASSO coefficient profiles of the 20 hub genes. A vertical line is drawn at the value chosen through 10-fold cross-validation. Red text indicates genes associated with prognosis obtained by LASSO COX regression.

the specific mechanism of this gene involved in endometrial carcinoma is not clear (59). Kuroda *et al* (60) have demonstrated that *TNNT1* is highly expressed in cancer tissues of the cervix, colon, lungs, ovaries and testes. Similarly, Gu *et al* (61) reported that postoperative recurrence of gallbladder carcinoma was associated with *TNNT1* expression, and *TNNT1* has been identified as a favorable prognostic biomarker for this disease. The results of the analysis in the present study demonstrated that high expression of *TNNT1* was significantly associated with poor OS in patients with ES, consistent with the above findings. To the best of our knowledge, no published studies on *TNNT1* in ES are currently available. Therefore, further study of the molecular mechanism of *TNNT1* in ES is required to identify more efficient and sensitive prognostic molecular markers for ES.

TTN encodes a large abundant protein of striated muscle and is involved heart and skeletal muscle diseases (62,63). However, a limited number of studies on *TTN* in cancer are currently available. *TTN* has been reported to be mutated frequently in several tumor types, including lung squamous cell carcinoma and lung and colon adenocarcinoma (64). Whole exome sequence data have been previously used to estimate the gene mutation rate of *TTN*, which identified *TTN* mutations in colorectal cancer, suggesting that *TTN* mutations may serve a specific role in the occurrence or progression of colorectal cancer (65). Study of low-abundance transcriptomes is crucial for determining the molecular mechanisms of tumor progression, and Bizama *et al* (66) identified *TTN* as a novel marker for advanced gastric cancer by analyzing low-abundance transcriptomes. Yang *et al* (67) conducted a genotyping study and demonstrated that *TTN* was a potential biomarker for predicting the clinical prognosis of patients with hepatitis B virus-associated hepatocellular carcinoma. The Cancer Genome Atlas-based aggregation analysis revealed that the missense mutation of *TTN* was associated with good prognosis in lung squamous cell carcinoma (68). However, no studies are currently available on *TTN* in ES, but the LASSO COX regression and OS analysis results of the present study demonstrated that high expression of *TTN* was associated with poor prognosis of patients with ES and may serve an important role in the development of ES. However, whether *TTN* can be used as a prognostic biomarker for ES requires verification in further experiments.

MYL3 encodes myosin light chain 3 and is associated with cardiomyopathy (69,70). A previous study has demonstrated that *MYL3* combined with Ca^{2+} can promote muscle development and participate in the contractile process of striated muscle (71). In addition, *MYL3* appears to be involved in strength development and fine co-ordination of muscle contraction (72). In a study on zebrafish, Meder *et al* (73) revealed that the phosphorylation of the C-terminal serine residue of *MYL3* is of great significance for cardiac contraction. However, whether *MYL3* also serves a certain role in cancer has not been confirmed. The results of the present study demonstrated that a high expression of *MYL3* was associated with poor prognosis in patients with ES, and this result may help explain the molecular mechanism of ES.

TMOD1 is involved in the development, invasion and metastasis of various tumors, including esophageal cancer (74),

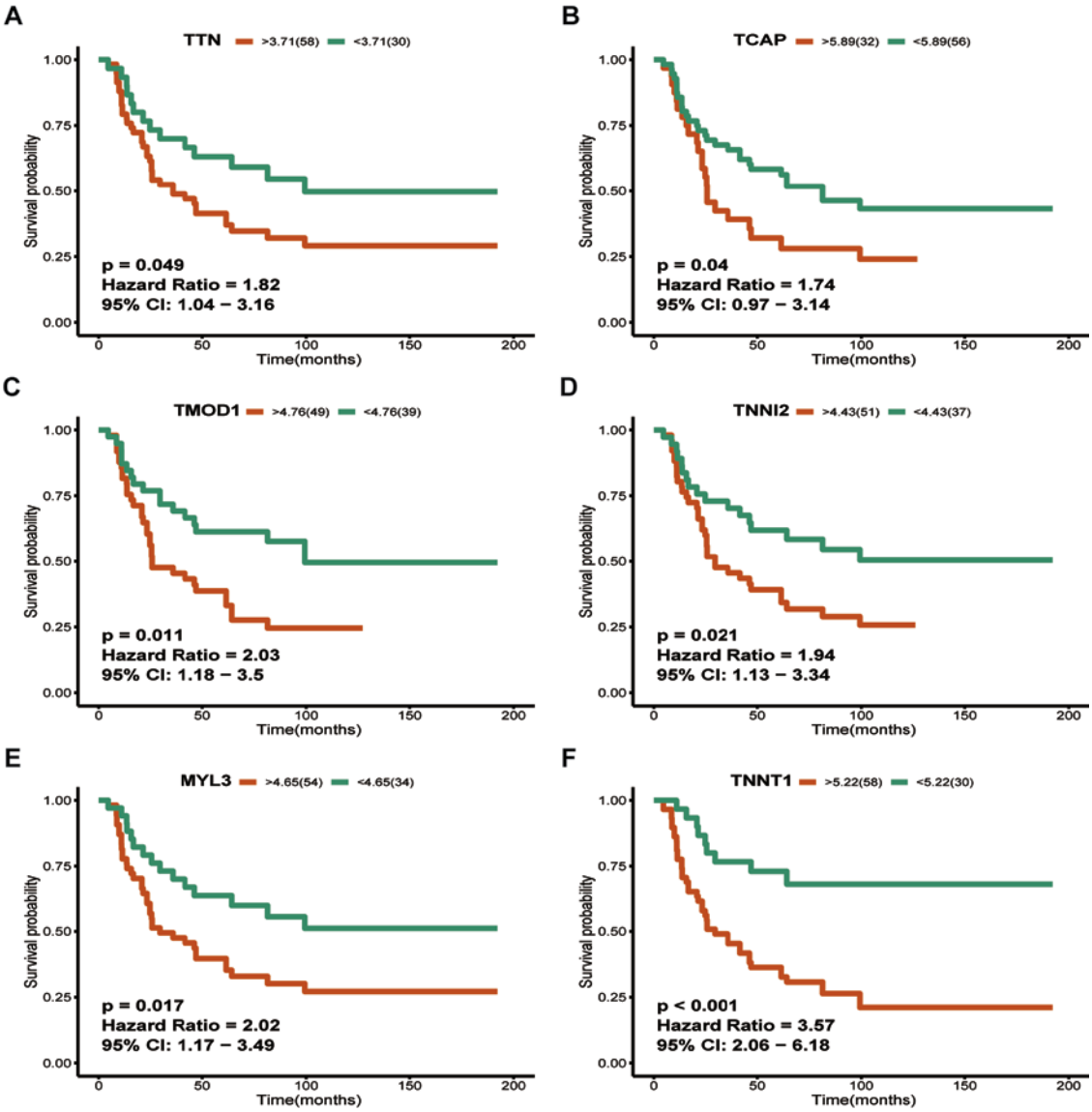

Figure 6. Kaplan-Meier curves. (A-F) OS curves of (A) *TTN*, (B) *TCAP*, (C) *TMOD1*, (D) *TNNI2*, (E) *MYL3* and (F) *TNNT1*. OS analysis was based on high and low expression of the genes using Best Separation. P<0.05 was considered to indicate a statistically significant difference. OS, overall survival; *TTN*, titin; *TCAP*, titin-cap; *TMOD1*, tropomodulin 1; *TTNI2*, fast skeletal type troponin I2; *MYL3*, myosin light-chain 3; *TNNT1*, slow skeletal muscle troponin T.

meningioma (75), breast cancer (76) and acute lymphocytic leukemia (77). High expression of *TMOD1* is a key biomarker for poor prognosis in patients with oral squamous cell carcinoma (78). *TCAP* is associated with the onset of breast cancer and is important for accurate diagnosis and prognosis assessment of breast cancer subtypes (79,80). Sawaki *et al* (81) analyzed mRNA expression in 15 gastric cancer cell lines and 262 surgically resected gastric tissues; the results demonstrated that the expression of *TNNI2* in gastric tissue can be used as a specific biomarker for predicting peritoneal metastasis of gastric cancer. The conclusions of the aforementioned studies support the results of the present study. However, owing to the lack of studies on ES, the credibility of the results of the present study needs to be verified in further experiments.

Previous studies have demonstrated that *TNNT1* is closely related to the invasion and metastasis of multiple types of tumors (56,58). High expression of *TNNT1* is a prognostic biomarker for a variety of cancers (48,59,61). However, there are a limited number of studies on the molecular mechanism of *TNNT1* in ES. The Rac1 pathway is associated with the malignant biological behavior of ES (40). Whether *TNNT1* is involved in the Rac1 pathway is still unclear; however, it may be hypothesized that *TNNT1* may participate in the invasion and metastasis of ES by activating the Rac1 pathway. Further molecular studies will be conducted in the future to validate these results.

The majority of bioinformatics studies focus on a single microarray data set and use a single method to analyze DEGs. In the present study, the raw data were derived from three mRNA microarray data sets, thus increasing the sample size and confidence. In addition, various analysis methods were applied to analyze the data in depth, which provided different perspectives. However, the present study has certain limitations. First, the data sets selected in the present study have certain heterogeneity. Although the batch data were removed and quality control and standardization were performed on the original data, a larger sample size and higher quality data sets are still required to verify the reliability of the results of this study. Second, the present study was a second mining and

analysis of previously published data sets. Although the results of several previous studies are consistent with the present results, further biological experiments are needed to verify the function of the identified DEGs in ES (82-85). Further molecular studies will be conducted in the future to clarify the specific molecular mechanisms of these DEGs in ES.

In conclusion, a total of 211 DEGs were identified by bioinformatics analysis of three mRNA microarray data sets, and these DEGs may serve key roles in the development of ES. Through further analysis, 20 Hub genes were screened and three genes (*TNNT1*, *TTN*, and *MYL3*) were obtained through LASSO COX regression and OS analysis. These three genes may be potential prognostic biomarkers for ES. These results provided a theoretical basis for elucidating the molecular mechanisms underlying ES development and identifying candidate biomarkers for ES, which may help in developing new strategies for the diagnosis and treatment of ES.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YJD, QQX, GZZ and JW conceived and designed the study. ZLW, SPL, XGH and YCG contributed to dataset selection and bioinformatics analysis. ZJM, YGW and XWK performed statistical analysis. YJD and QQX wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Orr WS, Denbo JW, Billups CA, Wu J, Navid F, Rao BN, Davidoff AM and Krasin MJ: Analysis of prognostic factors in extraosseous Ewing sarcoma family of tumors: Review of St. Jude Children's Research Hospital experience. Ann Surg Oncol 19: 3816-3822, 2012.
2. Fleuren ED, Versleijen-Jonkers YM, Boerman OC and van der Graaf WT: Targeting receptor tyrosine kinases in osteosarcoma and Ewing sarcoma: Current hurdles and future perspectives. Biochim Biophys Acta 1845: 266-276, 2014.
3. Zhang Z, Huang L, Yu Z, Chen X, Yang D, Zhan P, Dai M, Huang S, Han Z and Cao K: Let-7a functions as a tumor suppressor in Ewing's sarcoma cell lines partly by targeting cyclin-dependent kinase 6. DNA Cell Biol 33: 136-147, 2014.
4. Gaspar N, Hawkins DS, Dirksen U, Lewis IJ, Ferrari S, Le Deley MC, Kovar H, Grimer R, Whelan J, Claude L, *et al*: Ewing sarcoma: Current management and future approaches through collaboration. J Clin Oncol 33: 3036-3046, 2015.
5. Li Y, Shao G, Zhang M, Zhu F, Zhao B, He C and Zhang Z: miR-124 represses the mesenchymal features and suppresses metastasis in Ewing sarcoma. Oncotarget 8: 10274-10286, 2017.
6. Felgenhauer JL, Nieder ML, Krailo MD, Bernstein ML, Henry DW, Malkin D, Baruchel S, Chuba PJ, Sailer SL, Brown K, *et al*: A pilot study of low-dose anti-angiogenic chemotherapy in combination with standard multiagent chemotherapy for patients with newly diagnosed metastatic Ewing sarcoma family of tumors: A children's oncology group (COG) phase II study NCT00061893. Pediatr Blood Cancer 60: 409-414, 2013.
7. Haga A, Ogawara Y, Kubota D, Kitabayashi I, Murakami Y and Kondo T: Interactomic approach for evaluating nucleophosmin-binding proteins as biomarkers for Ewing's sarcoma. Electrophoresis 34: 1670-1678, 2013.
8. Scotlandi K, Avnet S, Benini S, Manara MC, Serra M, Cerisano V, Perdichizzi S, Lollini PL, De Giovanni C, Landuzzi L and Picci P: Expression of an IGF-I receptor dominant negative mutant induces apoptosis, inhibits tumorigenesis and enhances chemosensitivity in Ewing's sarcoma cells. Int J Cancer 101: 11-16, 2002.
9. Herrero-Martín D, Osuna D, Ordóñez JL, Sevillano V, Martins AS, Mackintosh C, Campos M, Madoz-Gúrpide J, Otero-Motta AP, Caballero G, *et al*: Stable interference of EWS-FLI1 in an Ewing sarcoma cell line impairs IGF-1/IGF-1R signalling and reveals TOPK as a new target. Br J Cancer 101: 80-90, 2009.
10. Landuzzi L, De Giovanni C, Nicoletti G, Rossi I, Ricci C, Astolfi A, Scopece L, Scotlandi K, Serra M, Bagnara GP, *et al*: The metastatic ability of Ewing's sarcoma cells is modulated by stem cell factor and by its receptor c-kit. Am J Pathol 157: 2123-2131, 2000.
11. Brohl AS, Solomon DA, Chang W, Wang J, Song Y, Sindiri S, Patidar R, Hurd L, Chen L, Shern JF, *et al*: The genomic landscape of the Ewing sarcoma family of tumors reveals recurrent STAG2 mutation. PLoS Genet 10: e1004475, 2014.
12. Wang YX, Mandal D, Wang S, Hughes D, Pollock RE, Lev D, Kleinerman E and Hayes-Jordan A: Inhibiting platelet-derived growth factor beta reduces Ewing's sarcoma growth and metastasis in a novel orthotopic human xenograft model. In Vivo 23: 903-909, 2009.
13. Do I, Araujo ES, Kalil RK, Bacchini P, Bertoni F, Unni KK and Park YK: Protein expression of KIT and gene mutation of c-kit and PDGFRs in Ewing sarcomas. Pathol Res Pract 203: 127-134, 2007.
14. Ahmed AA, Sherman AK and Pawel BR: Expression of therapeutic targets in Ewing sarcoma family tumors. Hum Pathol 43: 1077-1083, 2012.
15. Crompton BD, Stewart C, Taylor-Weiner A, Alexe G, Kurek KC, Calicchio ML, Kiezun A, Carter SL, Shukla SA, Mehta SS, *et al*: The genomic landscape of pediatric Ewing sarcoma. Cancer Discov 4: 1326-1341, 2014.
16. Lerman DM, Monument MJ, McIlvaine E, Liu XQ, Huang D, Monovich L, Beeler N, Gorlick RG, Marina NM, Womer RB, *et al*: Tumoral TP53 and/or CDKN2A alterations are not reliable prognostic biomarkers in patients with localized Ewing sarcoma: A report from the children's oncology group. Pediatr Blood Cancer 62: 759-765, 2015.
17. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, *et al*: NCBI GEO: Archive for functional genomics data sets-update. Nucleic Acids Res 41 (Database Issue): D991-D995, 2013.
18. Savola S, Klami A, Myllykangas S, Manara C, Scotlandi K, Picci P, Knuutila S and Vakkila J: High expression of complement component 5 (C5) at tumor site associates with superior survival in Ewing's sarcoma family of tumour patients. ISRN Oncol 2011: 168712, 2011.
19. Agelopoulos K, Richter GH, Schmidt E, Dirksen U, von Heyking K, Moser B, Klein HU, Kontny U, Dugas M, Poos K, *et al*: Deep sequencing in conjunction with expression and functional analyses reveals activation of FGFR1 in Ewing sarcoma. Clin Cancer Res 21: 4935-4946, 2015.

20. Gautier L, Cope L, Bolstad BM and Irizarry RA: affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20: 307-315, 2004.
21. Leek JT, Johnson WE, Parker HS, Jaffe AE and Storey JD: The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 28: 882-883, 2012.
22. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK: limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43: e47, 2015.
23. Yu G, Wang LG, Han Y and He QY: clusterProfiler: An R package for comparing biological themes among gene clusters. OMICS 16: 284-287, 2012.
24. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP: Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 102: 15545-15550, 2005.
25. Smoot ME, Ono K, Ruscheinski J, Wang PL and Ideker T: Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics 27: 431-432, 2011.
26. Doncheva NT, Assenov Y, Domingues FS and Albrecht M: Topological analysis and interactive visualization of biological networks and protein structures. Nat Protoc Nature protocols 7: 670-685, 2012.
27. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY: cytoHubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8 (Suppl 4): S11, 2014.
28. Durisová M and Dedik L: SURVIVAL-an integrated software package for survival curve estimation and statistical comparison of survival rates of two groups of patients or experimental animals. Methods Find Exp Clin Pharmacol 15: 535-540, 1993.
29. Unal I: Defining an optimal cut-point value in ROC analysis: An alternative approach. Comput Math Methods Med 2017: 3762651, 2017.
30. Van Mater D and Wagner L: Management of recurrent Ewing sarcoma: Challenges and approaches. Onco Targets Ther 12: 2279-2288, 2019.
31. Sandberg AA and Bridge JA: Updates on cytogenetics and molecular genetics of bone and soft tissue tumors: Ewing sarcoma and peripheral primitive neuroectodermal tumors. Cancer Genet Cytogenet 123: 1-26, 2000.
32. Chaber R, Łach K, Arthur CJ, Raciborska A, Michalak E, Ciebiera K, Bilska K, Drabko K and Cebulski J: Prediction of Ewing Sarcoma treatment outcome using attenuated tissue reflection FTIR tissue spectroscopy. Sci Rep 8: 12299, 2018.
33. Esiashvili N, Goodman M and Marcus RB Jr: Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: Surveillance epidemiology and end results data. J Pediatr Hematol Oncol 30: 425-430, 2008.
34. Bosma SE, Ayu O, Fiocco M, Gelderblom H and Dijkstra PDS: Prognostic factors for survival in Ewing sarcoma: A systematic review. Surg Oncol 27: 603-610, 2018.
35. Koppenhafer SL, Goss KL, Terry WW and Gordon DJ: mTORC1/2 and protein translation regulate levels of CHK1 and the sensitivity to CHK1 inhibitors in Ewing sarcoma cells. Mol Cancer Ther 17: 2676-2688, 2018.
36. Lin L, Huang M, Shi X, Mayakonda A, Hu K, Jiang YY, Guo X, Chen L, Pang B, Doan N, *et al*: Super-enhancer-associated MEIS1 promotes transcriptional dysregulation in Ewing sarcoma in co-operation with EWS-FLI1. Nucleic Acids Res 47: 1255-1267, 2019.
37. Henrich IC, Young R, Quick L, Oliveira AM and Chou MM: USP6 confers sensitivity to IFN-mediated apoptosis through modulation of TRAIL signaling in Ewing sarcoma. Mol Cancer Res 16: 1834-1843, 2018.
38. Xia L, Su X, Shen J, Meng Q, Yan J, Zhang C, Chen Y, Wang H and Xu M: ANLN functions as a key candidate gene in cervical cancer as determined by integrated bioinformatic analysis. Cancer Manag Res 10: 663-670, 2018.
39. Xia P and Xu XY: Prognostic significance of CD44 in human colon cancer and gastric cancer: Evidence from bioinformatic analyses. Oncotarget 7: 45538-45546, 2016.
40. Mendoza-Naranjo A, El-Naggar A, Wai DH, Mistry P, Lazic N, Ayala FR, da Cunha IW, Rodriguez-Viciana P, Cheng H, Tavares Guerreiro Fregnani JH, *et al*: ERBB4 confers metastatic capacity in Ewing sarcoma. EMBO Mol Med 5: 1087-1102, 2013.
41. Qin H and Liu W: MicroRNA-99a-5p suppresses breast cancer progression and cell-cycle pathway through downregulating CDC25A. J Cell Physiol 234: 3526-3537, 2019.
42. Wang J, Jia N, Lyv T, Wang C, Tao X, Wong K, Li Q and Feng W: Paired box 2 promotes progression of endometrial cancer via regulating cell cycle pathway. J Cancer 9: 3743-3754, 2018.
43. Zhao F, Zhou LH, Ge YZ, Ping WW, Wu X, Xu ZL, Wang M, Sha ZL and Jia RP: MicroRNA-133b suppresses bladder cancer malignancy by targeting TAGLN2-mediated cell cycle. J Cell Physiol 234: 4910-4923, 2019.
44. Zhang L, Kang W, Lu X, Ma S, Dong L and Zou B: LncRNA CASC11 promoted gastric cancer cell proliferation, migration and invasion in vitro by regulating cell cycle pathway. Cell Cycle 17: 1886-1900, 2018.
45. Guenther LM, Dharia NV, Ross L, Conway A, Robichaud AL, Catlett JL II, Wechsler CS, Frank ES, Goodale A, Church AJ, *et al*: A combination CDK4/6 and IGF1R inhibitor strategy for Ewing sarcoma. Clin Cancer Res 25: 1343-1357, 2019.
46. Lorin S, Borges A, Ribeiro Dos Santos L, Souquère S, Pierron G, Ryan KM, Codogno P and Djavaheri-Mergny M: c-Jun NH2-terminal kinase activation is essential for DRAM-dependent induction of autophagy and apoptosis in 2-methoxyestradiol-treated Ewing sarcoma cells. Cancer Res 69: 6924-6931, 2009.
47. Wilky BA, Kim C, McCarty G, Montgomery EA, Kammers K, DeVine LR, Cole RN, Raman V and Loeb DM: RNA helicase DDX3: A novel therapeutic target in Ewing sarcoma. Oncogene 35: 2574-2583, 2016.
48. Tong DL, Boocock DJ, Dhondalay GK, Lemetre C and Ball GR: Artificial neural network inference (ANNI): A study on gene-gene interaction for biomarkers in childhood sarcomas. PLoS One 9: e102483, 2014.
49. Cheung IY, Feng Y, Danis K, Shukla N, Meyers P, Ladanyi M and Cheung NK: Novel markers of subclinical disease for Ewing family tumors from gene expression profiling. Clin Cancer Res 13: 6978-6983, 2007.
50. Ohali A, Avigad S, Zaizov R, Ophir R, Horn-Saban S, Cohen IJ, Meller I, Kollender Y, Issakov J and Yaniv I: Prediction of high risk Ewing's sarcoma by gene expression profiling. Oncogene 23: 8997-9006, 2004.
51. Kikuta K, Tochigi N, Shimoda T, Yabe H, Morioka H, Toyama Y, Hosono A, Beppu Y, Kawai A, Hirohashi S and Kondo T: Nucleophosmin as a candidate prognostic biomarker of Ewing's sarcoma revealed by proteomics. Clin Cancer Res 15: 2885-2894, 2009.
52. Wei B and Jin JP: TNNT1, TNNT2, and TNNT3: Isoform genes, regulation, and structure-function relationships. Gene 582: 1-13, 2016.
53. Gupton SL, Anderson KL, Kole TP, Fischer RS, Ponti A, Hitchcock-DeGregori SE, Danuser G, Fowler VM, Wirtz D, Hanein D and Waterman-Storer CM: Cell migration without a lamellipodium: Translation of actin dynamics into cell movement mediated by tropomyosin. J Cell Biol 168: 619-631, 2005.
54. Lees JG, Bach CT and O'Neill GM: Interior decoration: Tropomyosin in actin dynamics and cell migration. Cell Adh Migr 5: 181-186, 2011.
55. Nguyen TH and Barr FG: Therapeutic approaches targeting PAX3-FOXO1 and its regulatory and transcriptional pathways in rhabdomyosarcoma. Molecules 23: pii: E2798, 2018.
56. Kawabe S, Mizutani T, Ishikane S, Martinez ME, Kiyono Y, Miura K, Hosoda H, Imamichi Y, Kangawa K, Miyamoto K and Yoshida Y: Establishment and characterization of a novel orthotopic mouse model for human uterine sarcoma with different metastatic potentials. Cancer Lett 366: 182-190, 2015.
57. Davidson B, Abeler VM, Forsund M, Holth A, Yang Y, Kobayashi Y, Chen L, Kristensen GB, Shih IeM and Wang TL: Gene expression signatures of primary and metastatic uterine leiomyosarcoma. Hum Pathol 45: 691-700, 2014.
58. Shi Y, Zhao Y, Zhang Y, AiErken N, Shao N, Ye R, Lin Y and Wang S: TNNT1 facilitates proliferation of breast cancer cells by promoting G_1/S phase transition. Life Sci 208: 161-166, 2018.
59. Lawrenson K, Pakzamir E, Liu B, Lee JM, Delgado MK, Duncan K, Gayther SA, Liu S, Roman L and Mhawech-Fauceglia P: Molecular analysis of mixed endometrioid and serous adenocarcinoma of the endometrium. PLoS One 10: e0130909, 2015.
60. Kuroda T, Yasuda S, Nakashima H, Takada N, Matsuyama S, Kusakawa S, Umezawa A, Matsuyama A, Kawamata S and Sato Y: Identification of a gene encoding slow skeletal muscle troponin t as a novel marker for immortalization of retinal pigment epithelial cells. Sci Rep 7: 8163, 2017.
61. Gu X, Li B, Jiang M, Fang M, Ji J, Wang A, Wang M, Jiang X and Gao C: RNA sequencing reveals differentially expressed genes as potential diagnostic and prognostic indicators of gallbladder carcinoma. Oncotarget 6: 20661-20671, 2015.

62. Watanabe H, Atsuta N, Hirakawa A, Nakamura R, Nakatochi M, Ishigaki S, Iida A, Ikegawa S, Kubo M, Yokoi D, *et al*: A rapid functional decline type of amyotrophic lateral sclerosis is linked to low expression of TTN. J Neurol Neurosurg Psychiatry 87: 851-858, 2016.
63. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, *et al*: Truncations of titin causing dilated cardiomyopathy. N Engl J Med 366: 619-628, 2012.
64. Kim N, Hong Y, Kwon D and Yoon S: Somatic mutaome profile in human cancer tissues. Genomics Inform 11: 239-244, 2013.
65. Wolff RK, Hoffman MD, Wolff EC, Herrick JS, Sakoda LC, Samowitz WS and Slattery ML: Mutation analysis of adenomas and carcinomas of the colon: Early and late drivers. Genes Chromosomes Cancer 57: 366-376, 2018.
66. Bizama C, Benavente F, Salvatierra E, Gutiérrez-Moraga A, Espinoza JA, Fernández EA, Roa I, Mazzolini G, Sagredo EA, Gidekel M and Podhajcer OL: The low-abundance transcriptome reveals novel biomarkers, specific intracellular pathways and targetable genes associated with advanced gastric cancer. Int J Cancer 134: 755-764, 2014.
67. Yang CK, Yu TD, Han CY, Qin W, Liao XW, Yu L, Liu XG, Zhu GZ, Su H, Lu SC, *et al*: Genome-wide association study of MKI67 expression and its clinical implications in HBV-related hepatocellular carcinoma in Southern China. Cell Physiol Biochem 42: 1342-1357, 2017.
68. Cheng X, Yin H, Fu J, Chen C, An J, Guan J, Duan R, Li H and Shen H: Aggregate analysis based on TCGA: TTN missense mutation correlates with favorable prognosis in lung squamous cell carcinoma. J Cancer Res Clin Oncol 145: 1027-1035, 2019.
69. Caleshu C, Sakhuja R, Nussbaum RL, Schiller NB, Ursell PC, Eng C, De Marco T, McGlothlin D, Burchard EG and Rame JE: Furthering the link between the sarcomere and primary cardiomyopathies: Restrictive cardiomyopathy associated with multiple mutations in genes previously associated with hypertrophic or dilated cardiomyopathy. Am J Med Genet A 155A: 2229-2235, 2011.
70. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, *et al*: Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): A policy statement of the American college of medical genetics and genomics. Genet Med 19: 249-255, 2017.
71. Grabarek Z: Structural basis for diversity of the EF-hand calcium-binding proteins. J Mol Biol 359: 509-525, 2006.
72. Morano I: Tuning smooth muscle contraction by molecular motors. J Mol Med (Berl) 81: 481-487, 2003.
73. Meder B, Laufer C, Hassel D, Just S, Marquart S, Vogel B, Hess A, Fishman MC, Katus HA and Rottbauer W: A single serine in the carboxyl terminus of cardiac essential myosin light chain-1 controls cardiomyocyte contractility in vivo. Circ Res 104: 650-659, 2009.
74. Gharahkhani P, Fitzgerald RC, Vaughan TL, Palles C, Gockel I, Tomlinson I, Buas MF, May A, Gerges C, Anders M, *et al*: Genome-wide association studies in oesophageal adenocarcinoma and Barrett's oesophagus: A large-scale meta-analysis. Lancet Oncol 17: 1363-1373, 2016.
75. Dalan AB, Gulluoglu S, Tuysuz EC, Kuskucu A, Yaltirik CK, Ozturk O, Ture U and Bayrak OF: Simultaneous analysis of miRNA-mRNA in human meningiomas by integrating transcriptome: A relationship between PTX3 and miR-29c. BMC Cancer 17: 207, 2017.
76. Ito-Kureha T, Koshikawa N, Yamamoto M, Semba K, Yamaguchi N, Yamamoto T, Seiki M and Inoue J: Tropomodulin 1 expression driven by NF-κB enhances breast cancer growth. Cancer Res 75: 62-72, 2015.
77. Núñez-Enríquez JC, Bárcenas-López DA, Hidalgo-Miranda A, Jiménez-Hernández E, Bekker-Méndez VC, Flores-Lujano J, Solis-Labastida KA, Martínez-Morales GB, Sánchez-Muñoz F, Espinoza-Hernández LE, *et al*: Gene expression profiling of acute lymphoblastic leukemia in children with very early relapse. Arch Med Res 47: 644-655, 2016.
78. Suzuki T, Kasamatsu A, Miyamoto I, Saito T, Higo M, Endo-Sakamoto Y, Shiiba M, Tanzawa H and Uzawa K: Overexpression of TMOD1 is associated with enhanced regional lymph node metastasis in human oral cancer. Int J Oncol 48: 607-612, 2016.
79. Staaf J, Jönsson G, Ringnér M, Vallon-Christersson J, Grabau D, Arason A, Gunnarsson H, Agnarsson BA, Malmström PO, Johannsson OT, *et al*: High-resolution genomic and expression analyses of copy number alterations in HER2-amplified breast cancer. Breast Cancer Res 12: R25, 2010.
80. Pan X, Hu X, Zhang YH, Chen L, Zhu L, Wan S, Huang T and Cai YD: Identification of the copy number variant biomarkers for breast cancer subtypes. Mol Genet Genomics 294: 95-110, 2019.
81. Sawaki K, Kanda M, Miwa T, Umeda S, Tanaka H, Tanaka C, Kobayashi D, Suenaga M, Hattori N, Hayashi M, *et al*: Troponin I2 as a specific biomarker for prediction of peritoneal metastasis in gastric cancer. Ann Surg Oncol 25: 2083-2090, 2018.
82. Postel-Vinay S, Véron AS, Tirode F, Pierron G, Reynaud S, Kovar H, Oberlin O, Lapouble E, Ballet S, Lucchesi C, *et al*: Common variants near TARDBP and EGR2 are associated with susceptibility to Ewing sarcoma. Nat Genet 44: 323-327, 2012.
83. Schmiedel BJ, Hutter C, Hesse M and Staege MS: Expression of multiple membrane-associated phospholipase A1 beta transcript variants and lysophosphatidic acid receptors in Ewing tumor cells. Mol Biol Rep 38: 4619-4628, 2011.
84. Foell JL, Hesse M, Volkmer I, Schmiedel BJ, Neumann I and Staege MS: Membrane-associated phospholipase A1 beta (LIPI) Is an Ewing tumour-associated cancer/testis antigen. Pediatr Blood Cancer 51: 228-234, 2008.
85. Kedage V, Selvaraj N, Nicholas TR, Budka JA, Plotnik JP, Jerde TJ and Hollenhorst PC: An interaction with Ewing's Sarcoma breakpoint protein EWS defines a specific oncogenic mechanism of ETS factors rearranged in prostate cancer. Cell Reports 17: 1289-1301, 2016.