JBUON 2018; 23(5): 1472-1480
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Identification of key genes and pathways in Ewing's sarcoma using bioinformatics analysis

Chen Yan[1]*, Yehua Wang[2]*, Qian Wang[3]*, Xiao Feng[2], Long Wang[2], Zan Bu[1], Bo Lu[1], Jian Jiang[2]

[1]Department of Orthopaedics, Xuzhou Medical University, Xuzhou, Jiangsu Province, China; [2]Department of Orthopaedics, the Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu Province, China; [3]Department of Rheumatology and Immunology, the Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu Province, China.

*Chen Yan, Yehua Wang and Qian Wang contributed equally to this work.

Summary

Purpose: *Ewing's sarcoma (ES) is a highly aggressive malignant bone and soft tissue neoplasm. The purpose of our study was to identify candidate biomarkers of ES and uncover their potential molecular mechanisms.*

Methods: *The gene expression profiles of GSE45544 and GSE73166 were downloaded from Gene Expression Omnibus (GEO) database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analyses were performed based on the Database for Annotation, Visualization and Integrated Discovery (DAVID) database, protein-protein interaction (PPI) network was constructed, and hub genes of the differentially expressed genes (DEGs) were identified by Cytoscape software.*

Results: *586 DEGs in total were identified in ES, and the top up-regulated genes were extremely potent as ES biomarkers. GO function analysis data showed that these DEGs were significantly enriched in DNA translation, nucleus, and protein binding. KEGG pathway analysis revealed that DEGs were enriched in the pathways involved in cancer, HTLV-I infection, viral carcinogenesis, ribosome, and cell cycle. PPI network showed that most of the hub genes were for ribosomal proteins, associated in some way with the biological process of ES.*

Conclusions: *In conclusion, we identified the DEGs and hub genes which could promote our comprehension of detailed mechanisms involved in the development of ES.*

Key words: *bioinformatics, biomarker, differentially expressed genes, Ewing's sarcoma, pathway*

Introduction

ES is a highly aggressive malignant bone and soft tissue neoplasm, which predominately influences children, adolescents and young adults. It is the second primary malignant bone tumor in young people, with common occurrence in the soft tissues of adults [1]. ES can arise in any bone of the body, and about 25% of the patients are diagnosed post-metastasis [2]. These patients, diagnosed with organ metastases have a < 30% disease-free survival (DFS) time after 5 years of follow-up [3,4]. Until now, the treatment of ES was mainly depended on eradication of the sarcoma; patients with a localized tumor experienced improved outcomes compared to those with metastasis over decades [5,6]. Besides, patients with localized tumor, who experienced initial remission, could suffer a relapse of ES months or years later. Unfortunately, therapy for relapsed ES is usually ineffective. Till to date, there

Correspondence to: Jian Jiang, MD. 99 Huaihai West Rd, Gulong District, Xuzhou, Jiangsu 221000, China.
Tel and Fax: +86 015162189543, E-mail: jiangjian01@yeah.net
Received: 27/03/2018; Accepted: 21/04/2018

is no reliable biomarker for early detection of ES in the clinic. Therefore, it is urgent to discover new biomarkers to screen ES at the earliest possible time. In addition, ES is a genetically complex and heterogeneous disease; its molecular mechanism and pathogenesis are still not clearly understood. Recently, the key genes associated with ES could be screened by microarray-based gene expression profiling technology. This enabled the investigation of molecular mechanisms involved in ES.

Microarray-based gene expression profiling could provide data on DEGs in the studied samples. By comparing gene expression profiles of ES tumor samples with those of normal (control) tissue samples, candidate biomarkers, functional in human ES tumors but not in control tissues, could be identified.

At first, we downloaded the original data (GSE45544 and GSE73166) from the GEO (http://www.ncbi.nlm.nih.gov/geo/). Gene expression profiles of sarcoma in patients with ES were compared with those in normal human tissues to identify the DEGs. These DEGs were subjected to GO, KEGG pathway enrichment analysis, and PPI network analysis. Based on the analyses of DEGs in biological functions and pathways, we aimed to identify potential candidate biomarkers to obtain deeper insights into the mechanisms of ES.

Methods

Microarray data preparation

GSE45544 and GSE73166 , based on GPL6244 platform (Affymetrix Human Gene 1.0 ST Array) , were used in this study. The gene expression profiles were downloaded from GEO database.

DEG identification

The raw data files used for this analysis included the CEL files (Affymetrix platform). Statistical software R (version 3.4.0, https://www.r-project.org/) and packages of Bioconductor (http://www.bioconductor.org/) were applied for significance analysis of DEGs between ES samples and normal tissue samples. Significant DEGs were selected based on ''limma'' package of Bioconductor. P<0.05 was considered statistically significant.

GO and KEGG pathway enrichment analysis

GO analysis is a common method for annotating genes [7] and KEGG analysis is a popular method for functional analyses of genes [8,9]. Biological analysis of DEGs was performed by GO enrichment, containing biological process (BP), cellular component (CC), and molecular function (MF). To analyze the effect of signaling pathway of these DEGs on function, KEGG pathway analysis was executed by the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool (https://david.ncifcrf.gov/) [10]. P<0.05 was considered statistically significant.

PPI network and module construction

Search Tool for the Retrieval of Interacting Genes (STRING) database is a very useful online tool to evaluate PPI information, providing systematic data regarding cellular processes [11]. In order to evaluate the interactive network relationships of the DEGs, we integrated and mapped them to STRING database; only a confidence score of 0.7 was selected as the cut-off criterion. Subsequently, PPI networks were constructed using the Cytoscape software [12]. In order to screen the clusters of original PPI network, module analysis by Molecular Complex Detection (MCODE) was carried out in Cytoscape software. The criteria for the network module screening were set beyond the number of nodes and MCODE scores (Degree Cutoff: 2, Node Score Cutoff: 0.2, K-Core: 2). Moreover, the function annotation and pathway enrichment analysis for DEGs were performed by DAVID software in the modules with a threshold of p<0.05.

Results

ES-related DEG identification

After quality evaluation of raw microarray data, we removed 6 cell line samples and 10 patient

Table 1. The most significantly up-regulated and down-regulated DEGs (top ten, ES versus normal tissue)

Probe set	*Gene symbol*	*Fold change*	*p value*
Up-regulated			
8111524	UGT3A2	15.39	3.33E-07
7908204	HMCN1	15.16	1.86E-09
8067869	RBM11	14.72	7.45E-08
8102200	DKK2	12.66	1.60E-08
7938329	SNORA23	12.26	6.74E-10
8096176	PTPN13	12.14	4.58E-09
8045688	TNFAIP6	12.11	1.22E-07
8069521	LIPI	11.43	1.97E-07
8021245	DCC	10.77	1.36E-07
8046536	HOXD10	10.35	7.00E-07
Down-regulated			
7907160	ATP1B1	-5.76	2.17E-07
8149927	CLU	-5.32	3.24E-06
8172204	MAOB	-5.31	1.64E-06
7935188	SORBS1	-5.25	3.19E-06
7944667	SORL1	-5.11	1.31E-08
8097080	SYNPO2	-4.94	5.61E-05
7903592	KIAA1324	-4.88	2.48E-04
7988414	GATM	-4.84	2.08E-05
8043438	IGKV2D-28	-4.43	2.64E-04
7983256	CKMT1B	-3.91	1.81E-04

samples with osteosarcoma, based on the GPL6244 platform. The total number of samples analyzed was 22 each of ES samples and control tissue samples. The GSE45544 dataset contained 14 patient and 22 normal tissue samples; GSE73166 dataset contained 8 patient tissue samples only. For statistical analysis, software R was used for preprocessing and differential gene expression analysis of microarray data. Using the $p<0.01$ and fold change >2.0 criteria, a total of 586 genes were identified from the analyses of GSE45544 and GSE73166, out of which, 447 were up-regulated and 139 down-regulated; the heat map is shown in Figure 1. The expression levels of the top 10 overexpressed and down-regulated DEGs are displayed in Table 1.

GO term analysis

We uploaded all DEGs to the online software DAVID to identify altered biological functions. GO analysis results showed that enriched terms of the DEGs were significantly focused on biological processes (BP), including DNA templated transcription, negative regulation of RNA polymerase II promoter-driven transcription, cell division, rRNA processing, and covalent chromatin modification (Figure 2A). In cell component (CC) part, the DEGs were enriched in nucleus, nucleoplasm, cytoplasm, and membranes (Figure 2B). The molecular function (MF) analysis further showed that these DEGs were mainly enriched in protein binding, poly (A) binding, DNA binding, and nucleic acid binding (Figure 2C).

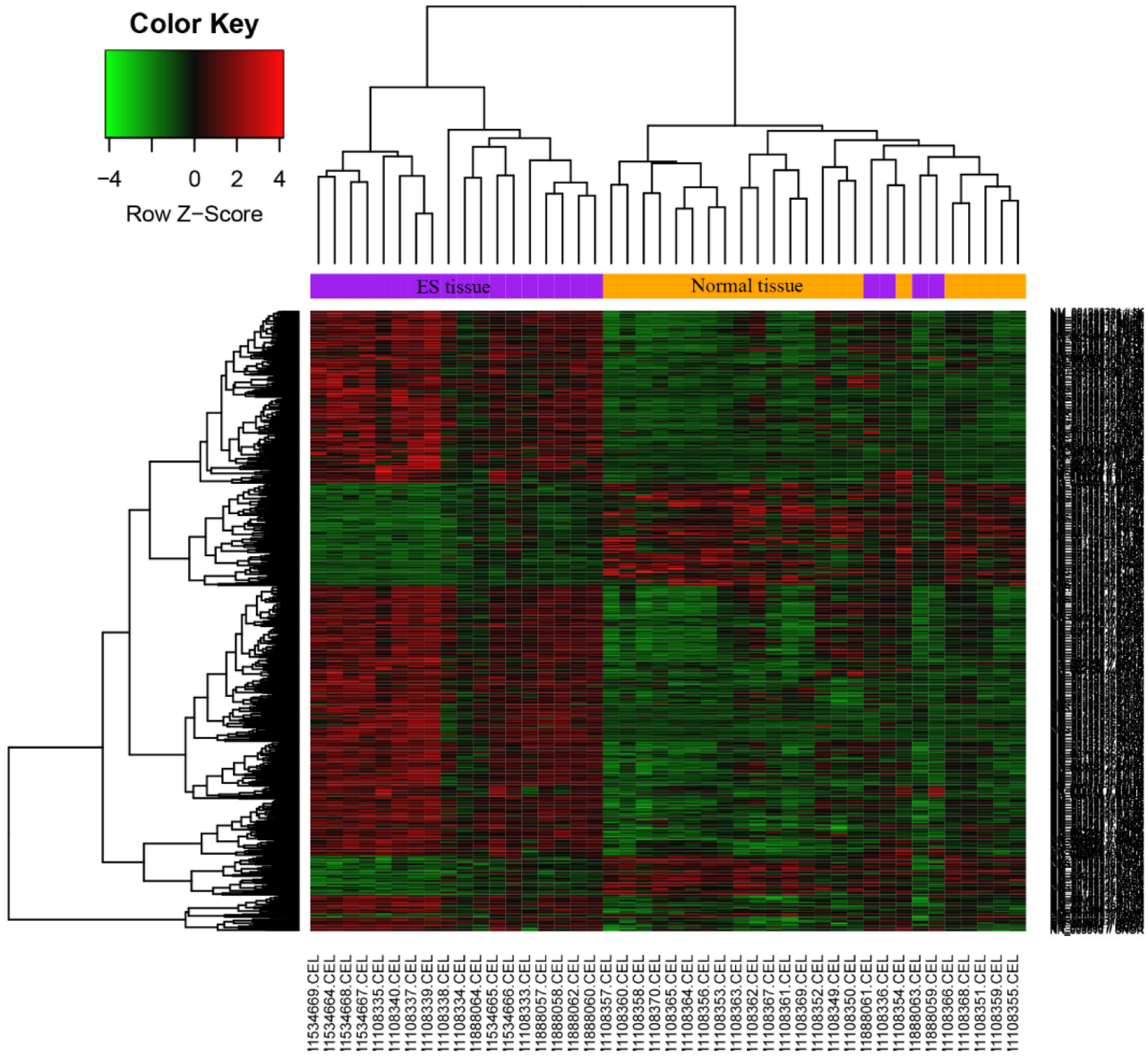

Figure 1. Heat map of differentially expressed genes (DEGs) of Ewing's sarcoma (ES) with fold change more than twice. Red, up-regulated DEGs; green, down-regulated DEGs; Purple, ES sample; Orange, normal sample.

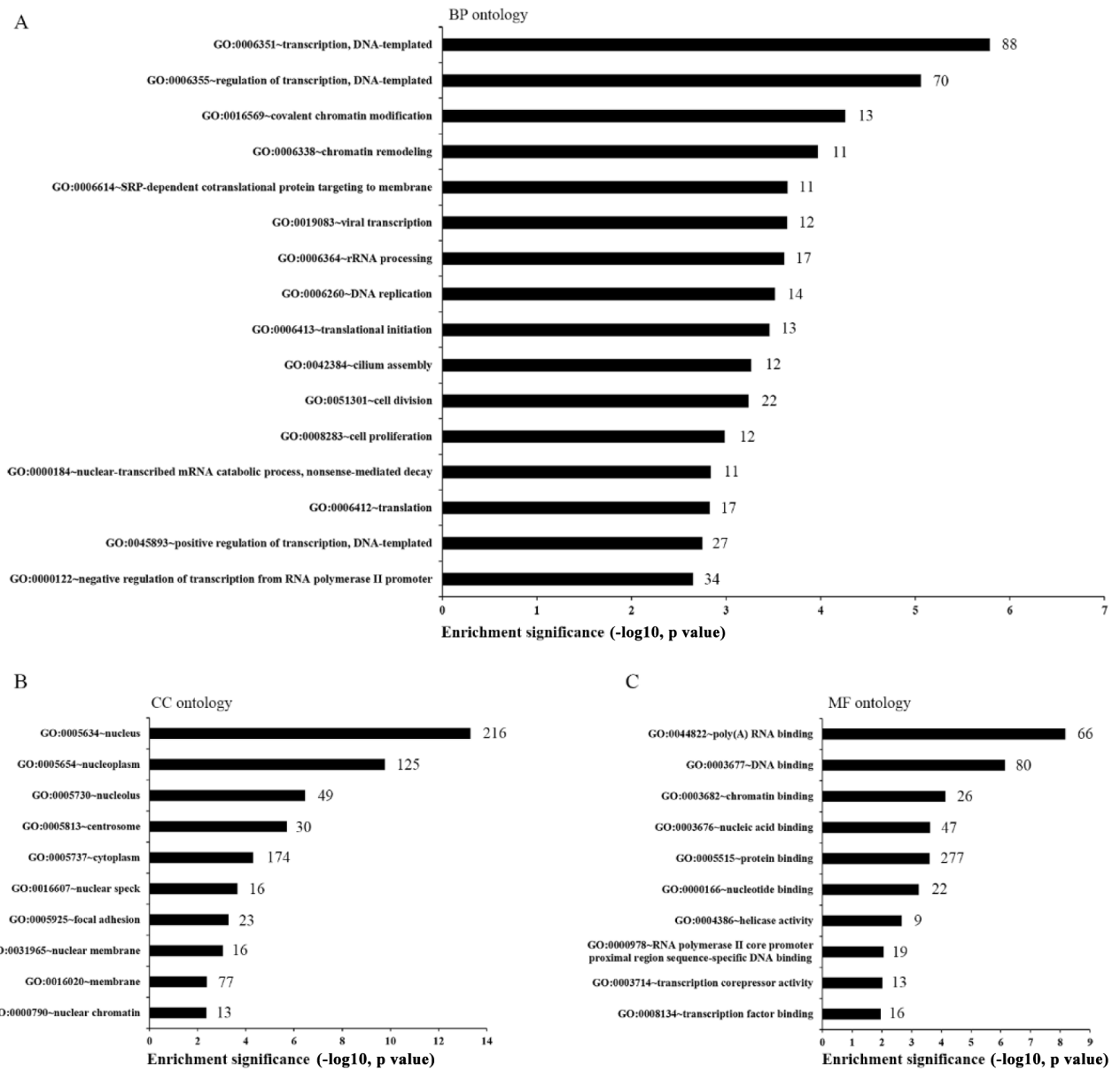

Figure 2. Gene Ontology (GO) enrichment of DEGs. **A:** BP, biological process; **B:** CC, cellular component; **C:** MF, molecular function. Count number was labeled in the list.

KEGG pathway analysis

The most significantly enriched pathways of DEGs, as obtained from KEGG analysis are listed in Table 2. The DEGs were mainly enriched in the pathways associated with cancer, HTLV-I infection, viral carcinogenesis, ribosome, and cell cycle.

Hub genes and PPI network module identification

STRING (a database of known and predicted protein interactions) is a method generally used to predict protein-protein interactions among the DEGs. The screened DEGs were uploaded to STRING to gain PPI data. In PPI networks, 9 nodal genes, including *RPS27, RPS2, RPS24, RPL23A, RPS19, RPL7, WDR12, RPL18A* and *NSA2* showed strong association with other nodal genes, suggesting higher degree for hubs (Figure 3A). These hub genes might have crucial effects on the development of ES.

Network module analysis

A significant module, obtained from PPI network using Cytoscape by default criteria, was further followed up with functional enrichment analysis (Figure 3B). The module chosen had 20 nodes with a MCODE score of 19. The GO function and KEGG pathway analysis of these hub genes are shown in Table 3. The module was mainly associated with translation, ribosome, and structural constituent of ribosome. This module was related

Table 2. KEGG pathway analysis of DEGs associated with ES

Pathway ID	*Name*	*Gene count*	*%*	*p value*	*Genes*
has05200	Cancer pathway	18	2.0	0.040	WNT5A, DCC, EGFR, CKS1B, LPAR4, SKP2, BCL2L1, CDK4, FZD4, DAPK1, HSP90B1, CCND1, HDAC2, JAK1, TPR, LAMB1, MYC, FN1
has05166	HTLV-I infection	15	1.7	0.010	WNT5A, KAT2A, XPO1, KAT2B, EGR2, SLC25A4, CHEK1, BCL2L1, CDK4, FZD4, CCND1, XBP1, JAK1, BUB1B, MYC
has05203	Viral carcinogenesis	12	1.4	0.025	KAT2A, CCND1, KAT2B, EGR2, HDAC2, GTF2H2C_2, GSN, SKP2, JAK1, CHEK1, ATP6V0D1, CDK4
has03010	Ribosome	11	1.3	0.004	RPL36A-HNRNPH2, RPS27, RPS19, RPL7, RPL6, RPLP0, RPL23A, RPL10A, RPL12, RPL39, RPS24
has04110	Cell cycle	9	1.0	0.020	CCND1, HDAC2, SKP2, BUB1B, PRKDC, CHEK1, CDK4, MYC, SMC3
has05222	Small cell lung cancer	8	0.9	0.008	CKS1B, CCND1, SKP2, BCL2L1, CDK4, LAMB1, MYC, FN1
has04919	Thyroid hormone signaling pathway	8	0.9	0.036	KAT2A, ATP1B1, CCND1, KAT2B, HDAC2, THRB, RCAN2, MYC
has03008	Ribosome biogenesis in eukaryotes	7	0.8	0.031	WDR75, XPO1, NOP58, HEATR1, GNL2, WDR43, GNL3
has00480	Glutathione metabolism	6	0.7	0.012	GSTA4, GCLC, GPX3, GSTT1, GPX7, MGST2
has05219	Bladder cancer	5	0.6	0.025	EGFR, CCND1, CDK4, MYC, DAPK1
has04742	Taste transduction	5	0.6	0.034	TAS2R50, TAS2R14, TAS2R31, SCNN1G, TAS2R20
has00330	Arginine and proline metabolism	5	0.6	0.047	CKMT1B, GATM, MAOB, ALDH2, OAT
has03430	Mismatch repair	4	0.5	0.024	EXO1, RFC4, RFC1, MLH3
has04320	Dorso-ventral axis formation	4	0.5	0.036	EGFR, CPEB2, CPEB3, CPEB4

to homologous recombination during KEGG pathway analysis.

Discussion

In the present study we identified significant DEGs by comparing human ES tissue with normal tissues through reanalysis of GSE45544 and GSE73166, downloaded from GEO database. To further analyze the roles of these DEGs, bioinformatics analyses including GO and KEGG pathway were performed using DAVID database, PPI network, and module construction by Cytoscape software.

A series of DEGs, namely *HMCN1, RBM11, DKK2, SNORA23, PTPN13* and *TNFAIP6* exhibited more than remarkable 12-fold change in the ES group than in the control group. Thus, we hypothesized that these DEGs might have the potential to be used as biomarkers for diagnosing ES, but only after further experimental confirmation in the future. *HMCN1* was reported to be significantly up-regulated in the ES and fibromatosis samples [13]. Though *RBM11* (RNA binding motif 11) has no such report, another RNA binding motif 3 was reported to participate in cancer. *RBM3* was overexpressed in esophageal and gastric adenocarcinoma, which might independently predict tumor recurrence [14]. *Dickkopf-2* (*DKK2*) could increase tumor vascular density and perfusion, and coverage of blood vessels by pericytes [15]. *SNORA23* (small nucleolar noncoding RNA 23) was overexpressed in human pancreatic ductal adenocarcinoma and correlated with patients' survival [16]. *PTPN13* is a new candidate tumor-suppressing gene in human cancer, negatively correlated with tumor grade and stage [17,18]. As already known, *TNF alpha* takes part in cancer development [19,20]. Serum *TNFAIP6* (TNF alpha-induced protein 6) was reported as a potential biomarker for Crohn's disease and ulcerative colitis [21]. Taken together, these DEGs are potent biomarkers to diagnose ES, provided further validations are performed.

Table 3. GO and KEGG pathway analyses of the module from PPI network

Category	*Term*	*p value*	*Genes*
Module			
BP ontology	Translation	6.35E-14	RPS27, RPS19, RPL18A, RPL13A, RPL6, RPL3, RPL23A, RPL10A, RPL12, RPS2, RPL39, RPS24
	Ribosomal large subunit assembly	1.40E-05	RPL6, RPL3, RPL23A, RPL12
	Regulation of translational initiation	5.34E-04	EIF3E, EIF4A2, EIF4A1
	Cytoplasmic translation	0.001	RPL7, RPL6, RPLP0
CC ontology	Ribosome	1.66E-14	RPS27, RPL18A, RPL7, RPL6, RPLP0, RPL3, RPLP2, RPL12, RPL39, RPS24
	Cytosolic large ribosomal subunit	1.45E-10	RPL7, RPL13A, RPL6, RPLP0, RPL3, RPL23A, RPL10A, RPL12
	Nucleolus	6.07E-04	RPS19, RPL7, RPL23A, RPL10A, SRP72, NSA2
	Membrane	0.005	RPS19, RPL7, EIF3E, RPLP0, EIF4A1, RPL10A
	Focal adhesion	0.006	RPS19, RPL7, RPLP0, RPL10A
MF ontology	Structural constituent of ribosome	6.77E-19	RPLP2, RPL23A, RPS2, RPL39, RPS27, RPS19, RPL7, RPL18A, RPL13A, RPL6, RPLP0, RPL3, RPL12, RPL10A, RPS24
	Poly(A) RNA binding	1.76E-06	RPS19, RPL7, EIF3E, EIF4A2, RPLP0, EIF4A1, RPL23A, RPL10A, SRP72, NSA2
	Translation initiation factor activity	0.007	EIF3E, EIF4A2, EIF4A1
KEGG	Homologous recombination	0.02	NBN, RAD50

BP: biological process, CC: cellular component, MF: molecular function

We also analyzed the biological functions of the DEGs based on GO annotation in DAVID database. At first, the majority of the DEGs were found enriched in DNA-templated transcription and regulation of transcription from RNA polymerase II promoter by BP function analysis. The results indicated that ES occurrence may begin with an erroneous DNA transcription. Other enriched categories containing those associated with cell division, chromatin modification, and rRNA processing were also indicated. In CC category, our data revealed nucleus, nucleoplasm, and cytoplasm to be the most significant items. The results showed association of DEGs with DNA reproduction and their possible participation in various biological processes of ES. In MF ontology, the most enriched item was protein binding, followed by DNA-, RNA-, and nucleic acid binding.

From the PPI network, a number of hub proteins have been identified, namely RPS19, RPS24, RPS27, RPL2, RPL7, RPL18A, RPL23A, WDR12 and NSA2, of which, most are ribosomal proteins. A full ribosome contains one large (60S) and one small (40S) subunit. It regulates the major step of gene expression by providing the site for translation. Ribosome biogenesis should be highly coordinated owing to the high accuracy and speed of the protein synthesis process. Hence, any aberration at any step might lead to abnormal cellular phenotype [22]. Recently, ribosomal proteins were related to diverse extra-ribosomal functions, including those associated with DNA repair, cell cycle, cell proliferation, differentiation, and malignant transformation. Dysfunction and impairment of ribosomal proteins could lead to malignant tumor or hematological and cardiovascular diseases [23-26]. RPS27 is correlated with gastric cancer metastasis [27]. RPS19 deficiency could lead to macrocytic anemia and bone marrow failure in mouse model [28]. RPL6 may be a potential prognostic biomarker for patients with gastric cancer [29]. The loss of RPL29 occurs during regulation of angiogenesis [30] by reducing micro-vessel formation stimulated by vascular endothelial growth factor [31]. Modules established from PPI network showed that 80% (16/20) compliments were ribosomal proteins. This implies that the hub ribosomal proteins in the module may play important roles in ES.

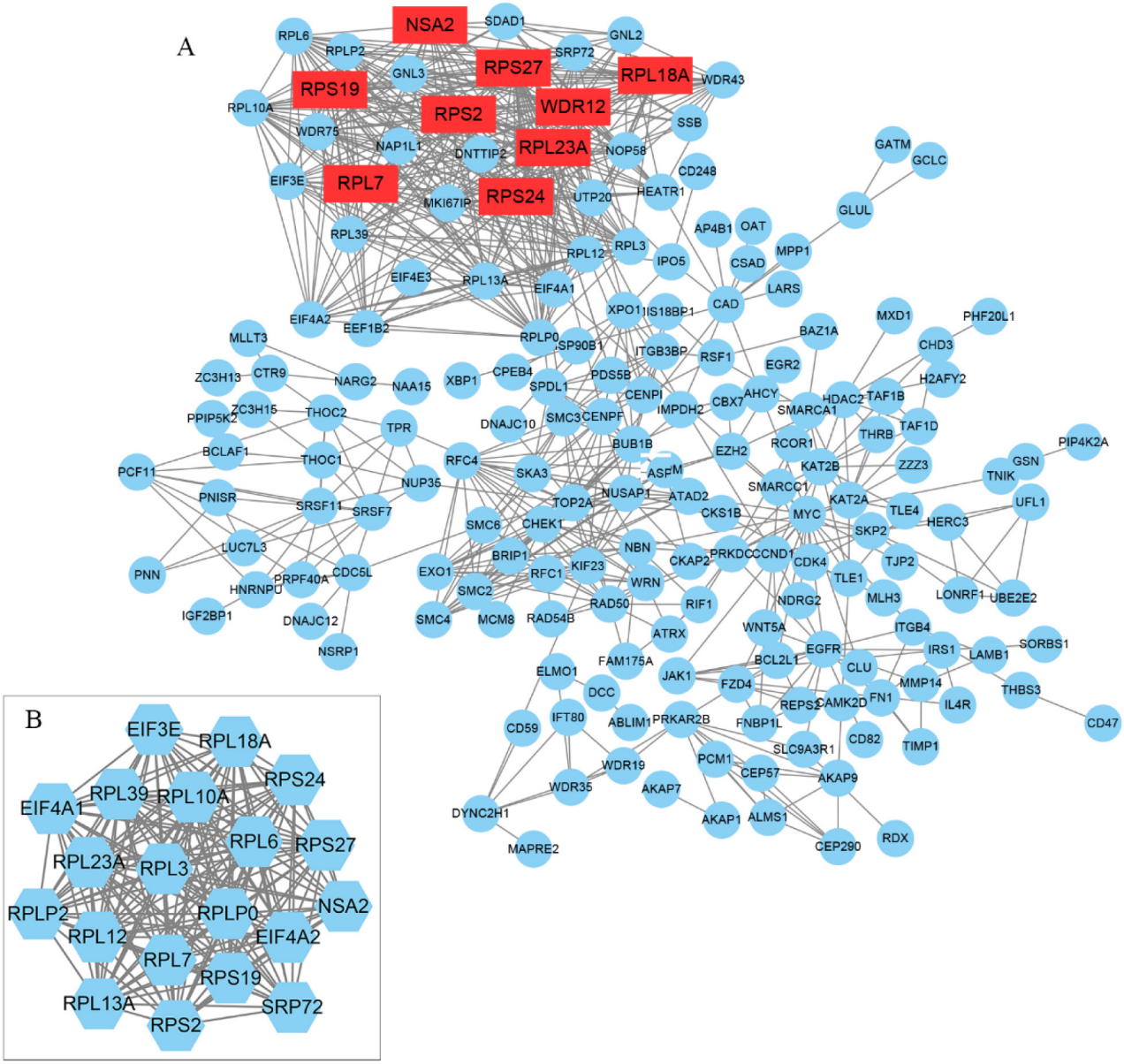

Figure 3. Protein-protein interaction network and module analysis. **A:** PPI network for DEGs; **B:** The module with the highest MCODE score.

The biological function of these module genes was further analyzed by GO annotation. In BP ontology, these module genes were enriched in gene translation and ribosomal large subunit assembly. In CC ontology, the main items were ribosome, cytosolic large ribosomal subunit, and nucleolus. In MF ontology, they focused on the structural constituents of ribosome. As the functions were mostly associated with ribosomal proteins, hence, we considered that the process of ES was correlated to the dysfunction of ribosomal proteins.

Pathway analysis of DEGs revealed 5 pathways that were mainly enriched: pathways in cancer, HTLV-1 (human T-cell leukemia virus type 1) infection, viral carcinogenesis, ribosome, and cell cycle. Obviously, general pathways involved in cancer would affect ES. Most hub genes/proteins, discussed above, are ribosomal genes/proteins, hence implying their role in ES by ribosome-specific pathway. HTLV-1 infection could result in adult T-cell leukemia/lymphoma that could group with viral carcinogenesis. Statistically, approximately 12% of human tumors, across the world, are caused by oncoviral infection. Oncoviral replication and persistence in human host were related to the activation of cancer-causing pathways. It was also reported that RPL18A interacts with nuclear/nucleolar protein to form a complex that retains viral mRNA in HTLV-1 infected cells [32]. Hence, it is comprehensible as to why HTLV-1 infection

and viral carcinogenesis influence the occurrence of ES.

Reports also showed that ribosomal proteins could serve as cancer biomarkers. RPL23A, RPL27, and RPL30 are increased in hepatocellular carcinoma, while RPL6 and RPL15 are up-regulated in gastric cancer [29,33]. RPL6 [29] and RPL23 [34] were suggested as candidate prognostic indicators of cancer and RPS2 could serve as therapeutic molecular target in prostate cancer [35]. Recombinant RPL23a and RPL31 also possessed anticancer activities [36,37]. Overexpressed RPL6 was associated with drug resistance and its reduced expression level could revert the resistance [38]. The 9 hub genes in PPI network were overexpressed (doubled approximately) in ES than in normal tissues, which may be considered as a bunch of proteins participating in ES development. Based on these results, we speculate that these hub genes could be recognized as targets for the diagnosis or prediction of ES development, if validated by further investigations.

Conclusion

In summary, our data provide a comprehensive bioinformatics analysis of DEGs, which may be involved in the development of ES. Moreover, it also predicts a set of targets for future investigation into the molecular mechanisms and biomarkers of ES. However, further experiments are recommended to confirm the functions of the identified genes and validate our speculation.

Conflict of interests

The authors declare no conflict of interests.

References

1. Avramova B, Jordanova M, Konstantinov D, Hristozova I, Shtarbanov I, Bobev D. Comparison of the treatment results after conventional and myeloablative chemotherapy in patients with poor prognosis Ewing's sarcoma family tumors - single center experience. JBUON 2011;16:551-6.
2. Borinstein SC, Beeler N, Block JJ et al. A decade in banking Ewing sarcoma: A report from the children's oncology group. Front Oncol 2013;3:57.
3. Rodriguez-Galindo C, Spunt SL, Pappo AS. Treatment of Ewing sarcoma family of tumors: Current status and outlook for the future. Med Pediatr Oncol 2003;40:276-87.
4. Lopez-Guerra JL, Marquez-Vega C, Praena-Fernandez JM et al. Health related quality of life and late side effects of long-term survivors of Ewing's sarcoma of bone. JBUON 2011;16:528-36.
5. Grier HE, Krailo MD, Tarbell NJ et al. Addition of ifosfamide and etoposide to standard chemotherapy for Ewing's sarcoma and primitive neuroectodermal tumor of bone. N Engl J Med 2003;348:694-701.
6. Balamuth NJ, Womer RB. Ewing's sarcoma. Lancet Oncol 2010;11:184-92.
7. The Gene Ontology (GO) project in 2006. Nucleic Acids Res 2006;34:D322-6.
8. Altermann E, Klaenhammer TR. PathwayVoyager: Pathway mapping using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. BMC Genomics 2005;6:60.
9. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28:27-30.
10. Dennis GJ, Sherman BT, Hosack DA et al. DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 2003;4:P3.
11. Franceschini A, Szklarczyk D, Frankild S et al. STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013;41:D808-15.
12. Shannon P, Markiel A, Ozier O et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498-2504.
13. Sarver AE, Sarver AL, Thayanithy V, Subramanian S. Identification, by systematic RNA sequencing, of novel candidate biomarkers and therapeutic targets in human soft tissue tumors. Lab Invest 2015;95:1077-88.
14. Jonsson L, Hedner C, Gaber A et al. High expression of RNA-binding motif protein 3 in esophageal and gastric adenocarcinoma correlates with intestinal metaplasia-associated tumours and independently predicts a reduced risk of recurrence and death. Biomark Res 2014;2:11.
15. Park H, Jung HY, Choi HJ et al. Distinct roles of DKK1 and DKK2 in tumor angiogenesis. Angiogenesis 2014;17:221-34.
16. Cui L, Nakano K, Obchoei S et al. Small nucleolar noncoding RNA SNORA23, Up-Regulated in human pancreatic ductal adenocarcinoma, regulates expression of spectrin Repeat-Containing nuclear envelope 2 to promote growth and metastasis of xenograft tumors in mice. Gastroenterology 2017;153:292-306.
17. Han X, Xue L, Zhou L et al. The role of PTPN13 in invasion and metastasis of lung squamous cell carcinoma. Exp Mol Pathol 2013;95:270-5.
18. Yeh SH, Wu DC, Tsai CY et al. Genetic characterization of fas-associated phosphatase-1 as a putative tumor suppressor gene on chromosome 4q21.3 in hepatocellular carcinoma. Clin Cancer Res 2006;12:1097-1108.

19. Balkwill F. TNF-alpha in promotion and progression of cancer. Cancer Metastasis Rev 2006;25:409-16.
20. Wu Y, Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer 2010;102:639-44.
21. Yu Q, Zhang S, Wang H et al. TNFAIP6 is a potential biomarker of disease activity in inflammatory bowel disease. Biomark Med 2016;10:473-83.
22. Bursac S, Brdovcak MC, Donati G, Volarevic S. Activation of the tumor suppressor p53 upon impairment of ribosome biogenesis. Biochim Biophys Acta 2014;1842:817-30.
23. Wang W, Nag S, Zhang X et al. Ribosomal proteins and human diseases: Pathogenesis, molecular mechanisms, and therapeutic implications. Med Res Rev 2015;35:225-85.
24. Ruggero D, Pandolfi PP. Does the ribosome translate cancer? Nat Rev Cancer 2003;3:179-92.
25. Shenoy N, Kessel R, Bhagat TD et al. Alterations in the ribosomal machinery in cancer and hematologic disorders. J Hematol Oncol 2012;5:32.
26. Freed EF, Bleichert F, Dutca LM, Baserga SJ. When ribosomes go bad: Diseases of ribosome biogenesis. Mol Biosyst 2010;6:481-93.
27. Yang ZY, Jiang H, Qu Y et al. Metallopanstimulin-1 regulates invasion and migration of gastric cancer cells partially through integrin beta4. Carcinogenesis 2013;34:2851-60.
28. Jaako P, Flygare J, Olsson K et al. Mice with ribosomal protein S19 deficiency develop bone marrow failure and symptoms like patients with Diamond-Blackfan anemia. Blood 2011;118:6087-96.
29. Gou Y, Shi Y, Zhang Y et al. Ribosomal protein L6 promotes growth and cell cycle progression through upregulating cyclin E in gastric cancer cells. Biochem Biophys Res Commun 2010;393:788-93.
30. Jones DT, Lechertier T, Reynolds LE et al. Endogenous ribosomal protein L29 (RPL29): A newly identified regulator of angiogenesis in mice. Dis Model Mech 2013;6:115-24.
31. Neumann F, Krawinkel U. Constitutive expression of human ribosomal protein L7 arrests the cell cycle in G1 and induces apoptosis in Jurkat T-lymphoma cells. Exp Cell Res 1997;230:252-61.
32. Ghorbel S, Sinha-Datta U, Dundr M, Brown M, Franchini G, Nicot C. Human T-cell leukemia virus type I p30 nuclear/nucleolar retention is mediated through interactions with RNA and a constituent of the 60 S ribosomal subunit. J Biol Chem 2006;281:37150-8.
33. Wang H, Zhao LN, Li KZ, Ling R, Li XJ, Wang L. Overexpression of ribosomal protein L15 is associated with cell proliferation in gastric cancer. BMC Cancer 2006;6:91.
34. Wu L, Li X, Xu F et al. Over-expression of RPL23 in myelodysplastic syndromes is associated with apoptosis resistance of CD34+ cells and predicts poor prognosis and distinct response to CHG chemotherapy or decitabine. Ann Hematol 2012;91:1547-54.
35. Wang M, Hu Y, Stearns ME. RPS2: A novel therapeutic target in prostate cancer. J Exp Clin Cancer Res 2009;28:6.
36. Sun B, Hou YL, Hou WR, Zhang SN, Ding X, Su XL. CDNA cloning, overexpression, purification and pharmacologic evaluation for anticancer activity of ribosomal protein L23A gene (RPL23A) from the Giant Panda. Int J Mol Sci 2012;13:2133-47.
37. Su XL, Hou YL, Yan XH et al. Expression, purification, and evaluation for anticancer activity of ribosomal protein L31 gene (RPL31) from the giant panda (Ailuropoda melanoleuca). Mol Biol Rep 2012;39:8945-54.
38. Du J, Shi Y, Pan Y et al. Regulation of multidrug resistance by ribosomal protein l6 in gastric cancer cells. Cancer Biol Ther 2005;4:242-7.