Construction of a Prognostic Signature in Ewing's Sarcoma: Based On Metabolism-Related Genes

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Abstract

Objective: By combining the expression profiles of metabolism-related genes (MRGS) with clinical information, the expression quantities of MRGS and the influence on development and prognosis were systematically analyzed, so as to provide a theoretical basis for the clinical study on the prognosis of Ewing's sarcoma.

Methods: MRGs expression profiles of 64 patients with Ewing's sarcoma were obtained from the GEO dataset. Univariate Cox regression analysis was used to identify metabolization-related differentially expressed genes (DEGs) related with prognosis in Ewing's sarcoma patients. Then, multivariate Cox analysis was used to calculate novel prognostic markers based on metabolism-related DEGs. Finally, the new prognostic index was verified on the basis of the prognostic models.

Results: Univariate Cox regression analysis identified 20 metabolization-related DEGs, 9 of which were significantly associated with Ewing's sarcoma patients' overall survival. Subsequently, we used nine metabolism-related DEGs to construct metabolism-related prognostic signature for patients with Ewing's sarcoma. Based on the 9 DEGs regression coefficient, we put forward the formula of each patient's risk score, and then divided the patients into high-risk group and low-risk group. The results indicated that the survival rate and survival time were higher in the low-risk group and lower in the high-risk group. Multivariate Cox analysis showed that risk score index was indeed an independent prognostic factor for Ewing's sarcoma. In addition, the area under the receiver operating characteristic (ROC) curve for overall survival was 0.985. And a nomogram model was established.

Conclusion: The experimental results suggest that the 9 metabolism-related DEGs marker may be effective in predicting the prognosis of Ewing's sarcoma to some extent, helping to individualize treatment of patients at different risks.

Introduction

Ewing's sarcoma is fairly common in children and adolescents bone tumors, cancer rate ranked second [1]. This is a highly malignant tumor of unknown origin, with no clear etiology and pathogenesis at present [2]. Among childhood cancers, the incidence of Ewing's sarcoma is 1.4% [3]. Since scientists first proposed the concept and nature of Ewing's sarcoma in the 1920s, its clinical treatment has made great progress. According to the common treatment of cancer, the current treatment methods for Ewing's sarcoma mainly include surgery, chemotherapy and radiotherapy, etc. After long-term exploration and efforts, although the prognosis of patients with localized tumors is good, the survival rate of patients with tumors with strong metastatic ability is very low [4]. Clinically, there are many factors used to evaluate the prognosis of Ewing's sarcoma, including tumor stage, classification and subtype, etc. Although these clinicopathological features can evaluate tumor prognosis to a certain extent, they cannot provide accurate and detailed prognostic information. Therefore, this may affect the judgment of clinical prognosis of patients. Some high-risk patients may face tumor metastasis and spread due to untimely or

inadequate treatment. At the same time, those low-risk patients may damage certain functions of the body due to excessive treatment. This will greatly affect the treatment and rehabilitation of patients. Then, we are urgently eager need to find some innovative molecular markers related to the development and prognosis of Ewing's sarcoma to predict the prognosis accurately and effectively, so as to treat patients favorably and improve and perfect the prognosis of patients.

At present, studies have found that metabolism-related genes are closely related to the occurrence, development and prognosis of tumors. Cancerous cells undergo a gradual process of adaptation to metabolism, which allows tumor cells to grow and proliferate rapidly, thus supporting the occurrence and development of tumors [5]. That is to say, During the above adaptation process, the tumor will rewrite its own nutritional metabolism program to meet the energy metabolism and biosynthesis of cancerous cells [6]. In addition, scientists have put forward a term "Warburg effect" for the metabolism of tumor cells, that is, even under the conditions of sufficient oxygen supply, the glycolysis process of tumor cells will be stronger than that of the original cells [7]. One study proposed that the occurrence and prognosis of esophageal cancer are closely related to the methylation level of folate metabolism-related genes, and that low serum and cell folic acid levels are factors that promote the occurrence of esophageal cancer [8]. A study found and identified 13 differentially expressed MRGs related to the prognosis of gastric cancer, and established a metabolic model that can be used to judge the prognosis of gastric cancer patients [9].

At present, many studies have confirmed that metabolism is closely related to Ewing's sarcoma. For example, Tadashi Kondo [10] said that through proteomic analysis, they proposed a new mechanism for the occurrence and development of Ewing's sarcoma, that is, the EWS-FLI-1 transition, and pointed out that EWS-FLI-1 has a certain regulatory effect on IL-6 secretion, thereby promoting tumor growth and metastasis. The lack of EWS-FLI-1 can lead to the excessive secretion of IL-6 and other soluble factors, which activate STAT signaling in bystander cells that maintain EWS-FLI-1 expression, and these factors can prevent the spontaneous apoptosis of tumor cells [11]. However, most studies focused on the relationship between one or a few MRGs and the occurrence, development, or prognosis of Ewing's sarcoma. There is a lack of studies using MRGs expression profiles containing dozens or even hundreds of MRGs to screen and identify molecular markers related to the prognosis of Ewing's sarcoma.

In our study, we integrated the MRGS expression profile of patients with Ewing's sarcoma, and combined with clinical data to obtain prognostic-related metabolic genes, so as to construct a prognostic model of MRGs and calculate the patient's risk value. So we can systematically analyze the differences in the expression of MRGs and their influence on tumor prognosis. Therefore, the use of metabolic-related DEGs markers can effectively predict the prognosis of Ewing's sarcoma, thereby helping to individualize the treatment of patients in different risk states.

Material And Methods

Metabolism-related genes and GEO data acquisition

852 MRGs were obtained from GSEA database (http://www.gsea-msigdb.org/gsea/index.jsp). The expression levels and clinical correlation of MRGs were obtained from the Gene Expression Omnibus (GEO) dataset (https://www.ncbi.nlm.nih.gov/gds) (GSE17679), including 64 Ewing's samples and 18 normal samples [12].

Identification of differentially expressed genes

Identify differentially expressed genes (DEG) related to metabolism between tumor samples and non-tumor samples according to the following criteria: false discovery rate (FDR) < 0.05 and [log2 (fold change)] > 0.5. Univariate Cox regression analysis was used to determine the metabolic-related DEG related to patient survival. Correlogram of prognostic MRGs were plotted using "corrgram" package in R (v. 4.0.0), including heat map, volcano map and box map.

Functional enrichment

The DAVID database Online Enrichment Tool (https://david.ncifcrf.gov/tools.jsp/) was used for Gene Ontology (GO) and the Kyoto Gene and Genomic Encyclopedia (KEGG) pathway enrichment. After GO and KEGG analysis, we can understand the functions and pathways behind DEGs related to metabolism, that is, the biological significance of MRGs.

Construction of the MPI model

Multivariate Cox regression analysis combined with clinical data was used to identify metabolic genes related to prognosis. The expression of each autophagy gene related to prognosis is weighted by regression coefficient, and then combined. After that, a risk score formula was established for each patient based on the combined results, and the patient's risk value was calculated. According to the risk score formula and the risk value, with the median value as the demarcation point, all patients were divided into high-risk groups and low-risk groups.

Statistical analysis

The Kaplan-Meier survival curve was utilized to analyze and evaluate the survival difference between the two groups. The risk curve was used to compare the patient's risk score and survival time. ROC curve was used to test the accuracy of model prediction. Conduct univariate and multivariate independent prognostic analysis and clinical correlation analysis, including the influence of age and gender on prognosis. All statistical tests were bilateral, and P value < 0.05 was regarded statistically significant.

Results

Identification of differentially expressed MRGs

We finally obtained 70 differentially expressed MRGs, including 25 up-regulated and 45 down-regulated MRGs (Fig. 1A and 1B). In addition, a boxplot was visualized to show the expression pattern of 70

differentially expressed MRGs between Ewing's sarcoma and non-tumor tissue (Fig. 1C).

Functional enrichment of the differentially expressed MRGs

Functional enrichment analysis of 70 differentially expressed MRGs provides a biological understanding of these genes. GO enrichment shows that the biological process (BP) of differential genes is mainly involved in nucleotide biosynthetic process, nucleotide phosphate biosynthetic process, small molecule catabolic process; cell composition (CC) is mainly involved in mitochondrial matrix; and molecular function (MF) is mainly involved in coenzyme binding (Fig. 2A and 2B). KEGG enrichment shows that pathways of differentially expressed MRGs mainly involve pathways in purine metabolism, arginine and proline metabolism, tyrosine metabolism, starch and sucrose metabolism and fatty acid degradation (Fig. 2C and 2D).

Identification of prognostic MRGs

The forest map of hazard ratios showed the hazard ratios of 20 MRGs (Fig. 3A). MRGs with significant significance were further included in the subsequent multivariate analysis. A total of 20 genes were significantly associated with prognosis after multivariate analysis. In the forest map of hazard ratios, green indicates that the genes are positively associated with favorable prognosis in Ewing's sarcoma, including 8 genes (ACADSB, AGL, ALDH7A1, CYP26B1, ENPP1, ENPP3, KDSR, KMO), and red indicates that the genes are positively associated with poor prognosis in Ewing's sarcoma, including 12 genes (ACSL1, ALDH18A1, AMPD1, DNMT1, GAMT, IDH2, LPCAT1, MAOA, MIF, NME1, RRM2, TYMS). Correlogram showed prognostic MRGs intercorrelations (Fig. 3B). Among them, ENPP1 is strongly positively correlated with MAOA, IDH2, RRM2, NME1, TYMS. ENPP3 is strongly positively correlated with ALDH18A1. KMO is strongly positively correlated with MAOA. RRM2 is strongly negatively correlated with DNMT1, NME1, TYMS. And NME1 is strongly negatively correlated with AMPD1, TYMS.

Construction of Metabolic prognostic index

We pooled prognostic MRGs for multivariate Cox regression analysis and constructed Metabolic prognostic index (MPI). Patients were divided into two groups with risk score, which could be calculated based on the MPI. Figure 3C showed distribution of prognostic index, survival status and survival rate of patients of the two groups and heatmap of the expression profile of the included MRGs.

In order to determine the role of the MPI in predicting clinical outcomes in Ewing's sarcoma patients, K-M survival curves were plotted to analyze different survival times between high-risk and low-risk groups. K-M analysis showed that the survival rate of patients in the high-risk group was significantly lower than that in the low-risk group (Fig. 3D). Univariate analysis showed that MPI was significantly associated with patient prognosis (Fig. 4A). In addition, after adjusting for clinicopathological features such as gender, age and PRS type, MPI remained an independent prognostic indicator for Ewing's sarcoma patients in multivariate analysis (Fig. 4B). The area under the curve of the corresponding receiver operating

characteristic (ROC) curve for survival is 0.985. This indicated that the prognostic index based on MRGs has a certain role and potential in the prediction of prognosis. (Fig. 4C).

Clinical Correlation Analysis and Differential Expression of MRGs

Clinical correlation analyses compared risk scores for different ages and genders. The results showed that the risk score of < = 18 age group was lower than that of > 18 age group ($P < 0.05$) (Fig. 4D). And male risk scores were relatively higher than female risk scores ($P < 0.05$) (Fig. 4E). Figure 5 showed the expression levels of the above 9 MRGs significantly associated with prognosis in the high-risk and low-risk groups. The genes expressed more in the low-risk group than in the high-risk group were AGL, ALDH7A1, CYP26B1, ENPP1 and KDSR. On the contrary, the genes were AMPD1, GAMT, LPCAT1 and RRM2. Genes with high expression in the low-risk group were positively associated with a good prognosis. Genes with high expression in the high-risk group were positively associated with a poor prognosis.

Discussion

Ewing's sarcoma is a highly aggressive and metastatic tumor with a high incidence in children and young adults. The occurrence of Ewing's sarcoma requires multiple steps, which are related to the genetic and epigenetic changes of intracellular proto-oncogenes and tumor suppressor genes [13]. There are age and gender differences in Ewing's sarcoma. It mainly occurs in men aged 5–25, and 80% of them occur in men under 20 years of age [14]. The current treatment of Ewing’s sarcoma mainly includes chemotherapy, radiotherapy and surgical resection to control its occurrence [15]. Although a large number of studies have shown that metabolism is involved in the occurrence and development of Ewing's sarcoma, MRGS has not been comprehensively analyzed to explore its clinical application and significance.

To metabolically analyze Ewing's sarcoma prognosis-related genes, we screened and identified 70 differentially expressed MRGs and 20 prognostic MRGs. Our results suggested that a prognostic model based on 9 MRGs can be used to classify and stratify prognosis in patients with Ewing's sarcoma, thus facilitating the individualization of treatment plans based on patient risk. According to GO and KEGG analysis, we discovered that these MRGs influenced the prognosis of Ewing's sarcoma mainly through the following functions and pathways, including small molecule catabolic process, coenzyme binding, arginine and proline metabolism, tyrosine metabolism and fatty acid degradation. The role of the regulation of small-molecule metabolic processes, cofactor-binding, amino acid, proteasome and ribosome biosynthesis in eukaryotes in Ewing's sarcoma development and prognosis has been identified and validated by studies [16]. In the mitochondria of malignantly proliferating tumor cells, some necessary metabolic processes occur, such as the oxidative metabolism of glucose and glutamine to produce citrate and acetyl-CoA, which are involved in the synthesis of lipids. [17].

We screened and obtained valuable MRGs that can predict the prognosis of Ewing's sarcoma. Many previous studies have confirmed that some of these MRGs are closely related to the prognosis of Ewing's

sarcoma or other malignant tumors. OX40L transgenic Ewing sarcoma cells showed retained the expression of certain Ewing sarcoma-associated (anti)gens including lipase member I, CCND1, CYP26B1, and EWSR1-FLI1 oncogene [18]. Targeting the ATR, CHK1, and WEE1 kinases in Ewing sarcoma cells activates CDK2 and reduce the ability of DNA replication by promoting the proteasome-mediated degradation of RRM2 [19]. In addition, the translation inhibitor 4E-BP1 can regulate the expression level of RRM2, thereby reducing its expression, inhibiting the occurrence and development of tumors, and improving the prognosis of Ewing's sarcoma [20]. Both indicated an association between RRM2 and Ewing's sarcoma. For other tumors, a study elucidated the role of AGL deficiency in promoting the development of bladder cancer through the generation and characterization of genetically engineered mice [21]. More and more evidence showed that ALDH7A1, which is one of the superfamily members of ALDH, can degrade and detoxify acetaldehyde produced by cell metabolism, and thus affect the occurrence and prognosis of many cancers [22]. The expression level of AMPD1 in patients with papillary thyroid carcinoma is higher, which is positively correlated with the malignant proliferation of the tumor and the poor prognosis of the patient. Therefore, AMPD1 can be used as a clinical molecular marker to judge the prognosis of patients, and even become a new therapeutic target. [23]. ENPP1 has been found to be related to the stemness of tumor cells in cancers such as breast cancer and glioblastoma, and is involved in tumor occurrence, nausea growth and metastasis. Therefore, ENPP1 can also be used as a biomarker for the diagnosis of malignant tumors and used to predict the prognosis of patients. [24]. GAMT is mainly involved in the regulation of two kinds of apoptosis in tumorigenesis, namely p53-dependent cell apoptosis for genotoxic stress and cell apoptosis induced by glucose deprivation. Among them, the former enables cells to regulate their own metabolism and energy levels to choose apoptosis or survival in the case of nutrient deficiency [25]. Studies have pointed out that LPCAT1 is a gene necessary for the proliferation and spread of malignant tumors, and its high-level expression is positively correlated with the poor prognosis of patients. It also reveals the valuable prognostic role of the miR-205-LPCAT1 axis in a variety of cancers [26].

The above research still has limitations to a certain extent. First of all, our research is retrospective and needs to use existing data to conduct experiments, so statistical bias is unavoidable. Secondly, the prognostic model we have established is not mature and perfect, and needs further verification and improvement to ensure the reliability of the model. Third, our research is not comprehensive enough, and the potential mechanism of MRG's influence on prognosis needs further exploration and reveal.

In summary, this study comprehensively analyzed the expression profile and clinical data of MRGs, and determined the prognostic-related MRGs of Ewing's sarcoma. The metabolism-related DEGs marker may be effective in predicting the prognosis of Ewing's sarcoma to some extent, and helping to individualize treatment of patients at different risks.

Conclusion

In this study, we used a series of algorithms and analyses to develop prognostic index based on MRGs analysis in Ewing's sarcoma, and ultimately identified 70 differentially expressed MRGs and 20 differentially expressed MRGs affecting prognosis. The 9-MRGs markers can help judge the prognosis of Ewing's sarcoma clinically, thereby helping to individualize the treatment of patients in different groups, and provide a solid foundation for the treatment and prognosis of Ewing's sarcoma.

Abbreviation

Ewing’s sarcoma, ES;

Gene Expression Omnibus, GEO;

Differentially expressed gene, DEG;

Receiver operating characteristic curve, ROC curve;

Gastric cancer, GC;

Metabolism-related genes, MRGs;

False discovery rate, FDR;

Database for Annotation Visualization and Intergrated Discovery, DAVID;

Gene Ontology, GO;

Kyoto Gene and Genomic Encyclopedia, KEGG

Metabolic prognostic index, MPI;

Kaplan-Meier survival curve, K-M survival curve;

Biological process, BP;

Cell composition, CC;

Molecular function, MF;

Declarations

Conflict of interest

These is no conflict of interests.

Author contribution

WB designed the study and collected data. YB drafted the manuscript. HY, JY, WH contributed to the writing. ZD provided critical feedback and contributed to the review of the manuscript. All authors contributed to the article and approved the submitted version.

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Tables

Table 1 Multivariate Cox regression analysis of identified metabolism-related DEGs.

	Coef	HR	HR.95L	HR.95H	Pvalue
AGL	-13.8029	1.01E-06	3.98E-09	0.000258	1.04E-06
ALDH7A1	-13.6105	1.23E-06	2.65E-09	0.000569	1.39E-05
AMPD1	-6.39977	0.001662	7.09E-05	0.038935	6.98E-05
CYP26B1	-11.1935	1.38E-05	3.52E-07	0.000538	2.17E-09
ENPP1	4.28493	72.59747	6.883864	765.6154	0.000364
GAMT	32.16778	9.34E+13	3.29E+08	2.65E+19	5.14E-07
KDSR	7.054725	1158.319	47.45774	28271.54	1.51E-05
LPCAT1	12.18001	194854.2	775.2573	48974895	1.56E-05
RRM2	5.82334	338.0994	10.68222	10701.07	0.000954

Table 2 Univariate and multivariate Cox regression analysis for ES prognosis

	ID	HR	HR.95L	HR.95H	Pvalue
multiCox	gender	0.763417	0.390497	1.492470	0.429971
	age	0.991681	0.941825	1.044176	0.750913
	PRS type	1.292856	0.834920	2.001959	0.249616
	riskScore	1.001797	1.000839	1.002756	0.000236
uniCox	gender	0.790139	0.410652	1.520314	0.480558
	age	0.996650	0.950605	1.044925	0.889417
	PRS type	1.241552	0.827521	1.862733	0.295882
	riskScore	1.001727	1.000789	1.002666	0.000306

Figures

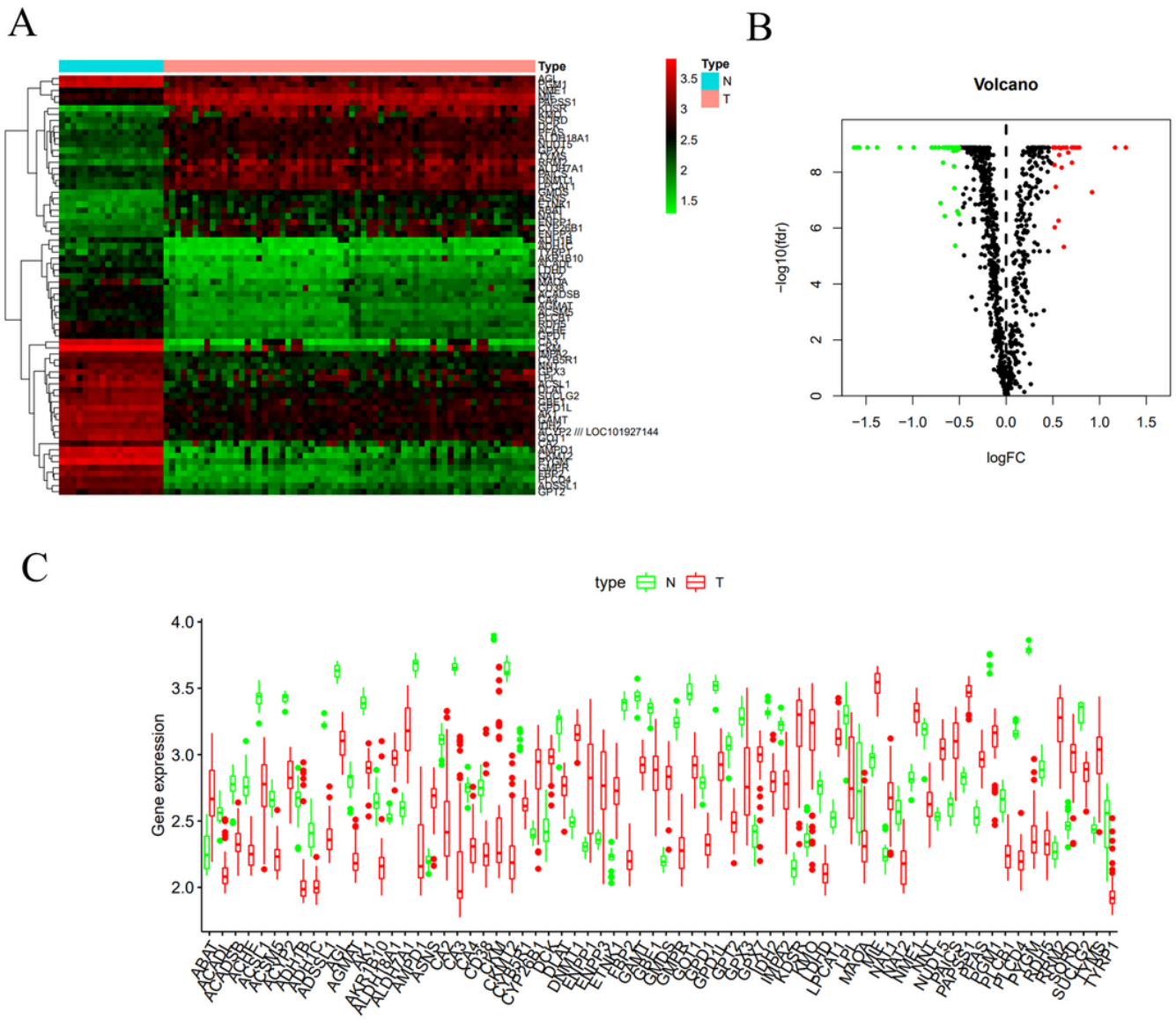

Figure 1

Metabolism-related differentially expressed genes (DEGs). Heat map(A) and volcanic map(B)showed DEGs between tumor samples and non-tumor samples. Red dots represented significantly up-regulated genes, green dots represented significantly down-regulated genes, while black dots indicated no differences. (C)Expression patterns of metabolism-related DEGs in tumor and non-tumor samples. The red block represented tumor samples and the blue block represented non-tumor samples.

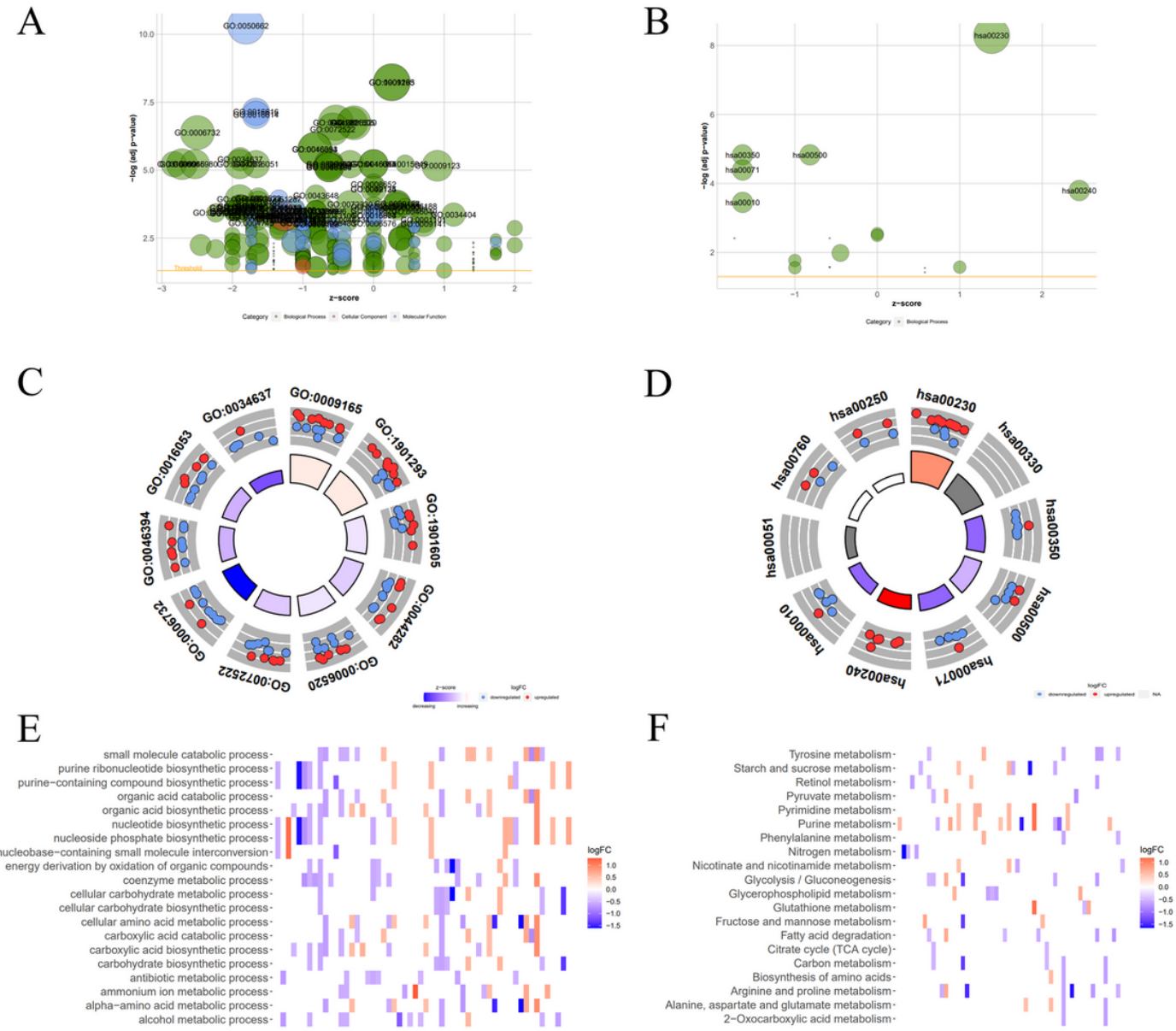

Figure 2

The bubble diagram, circle diagram and heatmap showed the GO functional enrichment of metabolism-related DEGs (A) (C) (E), and KEGG pathway enrichment of metabolism-related DEGs(B) (D) (F).

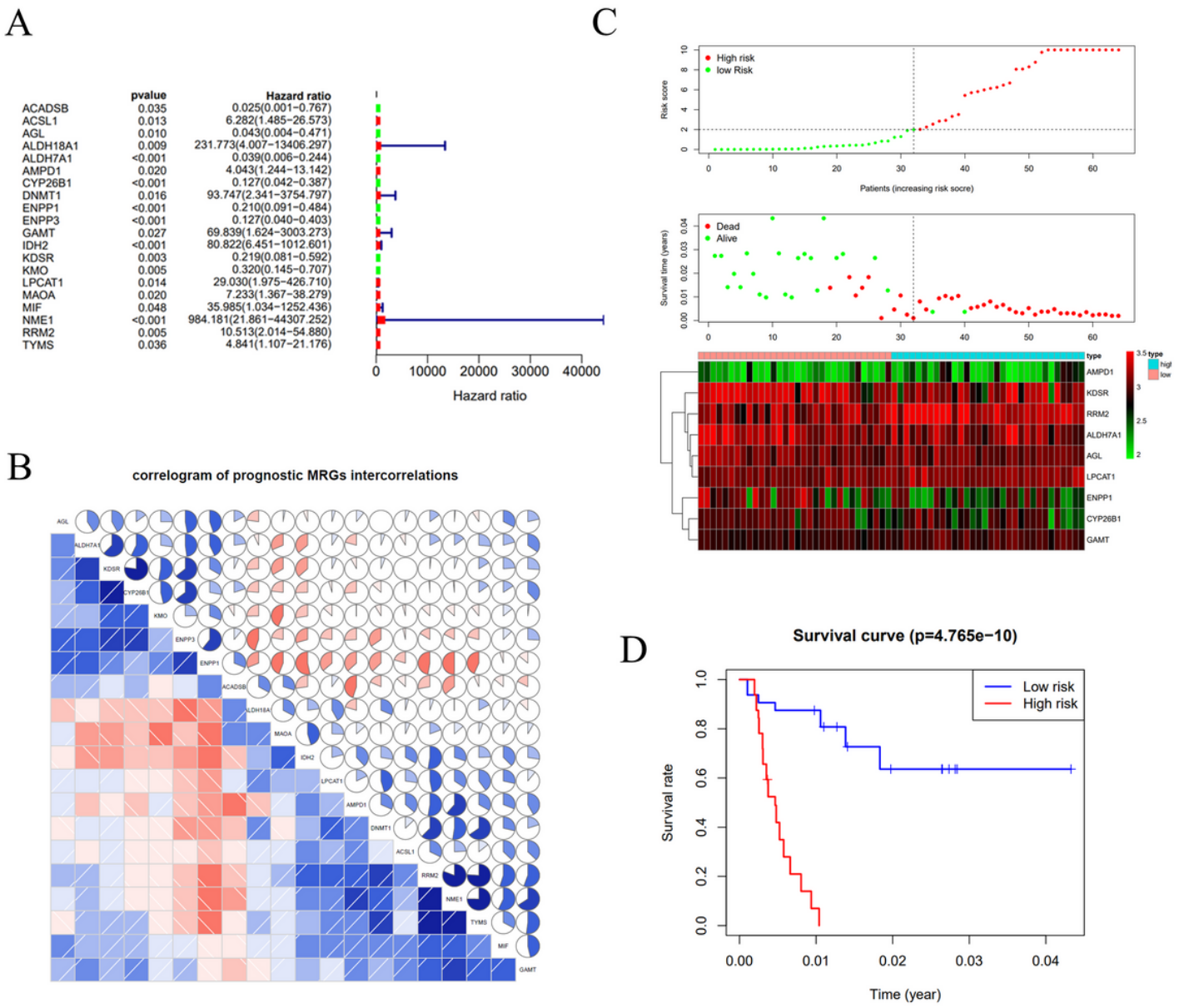

Figure 3

(A)The Hazard ratio forest map shows the significant prognostic value of the genes. (B) Correlogram of prognostic MRGs. (C) Survival status of patients in the high RiskScore and low RiskScore groups; Expression spectrum of identified genes in high RiskScore and low RiskScore groups. (D) Kaplan-Meier survival curve analysis of survival differences between high RiskScore and low RiskScore groups.

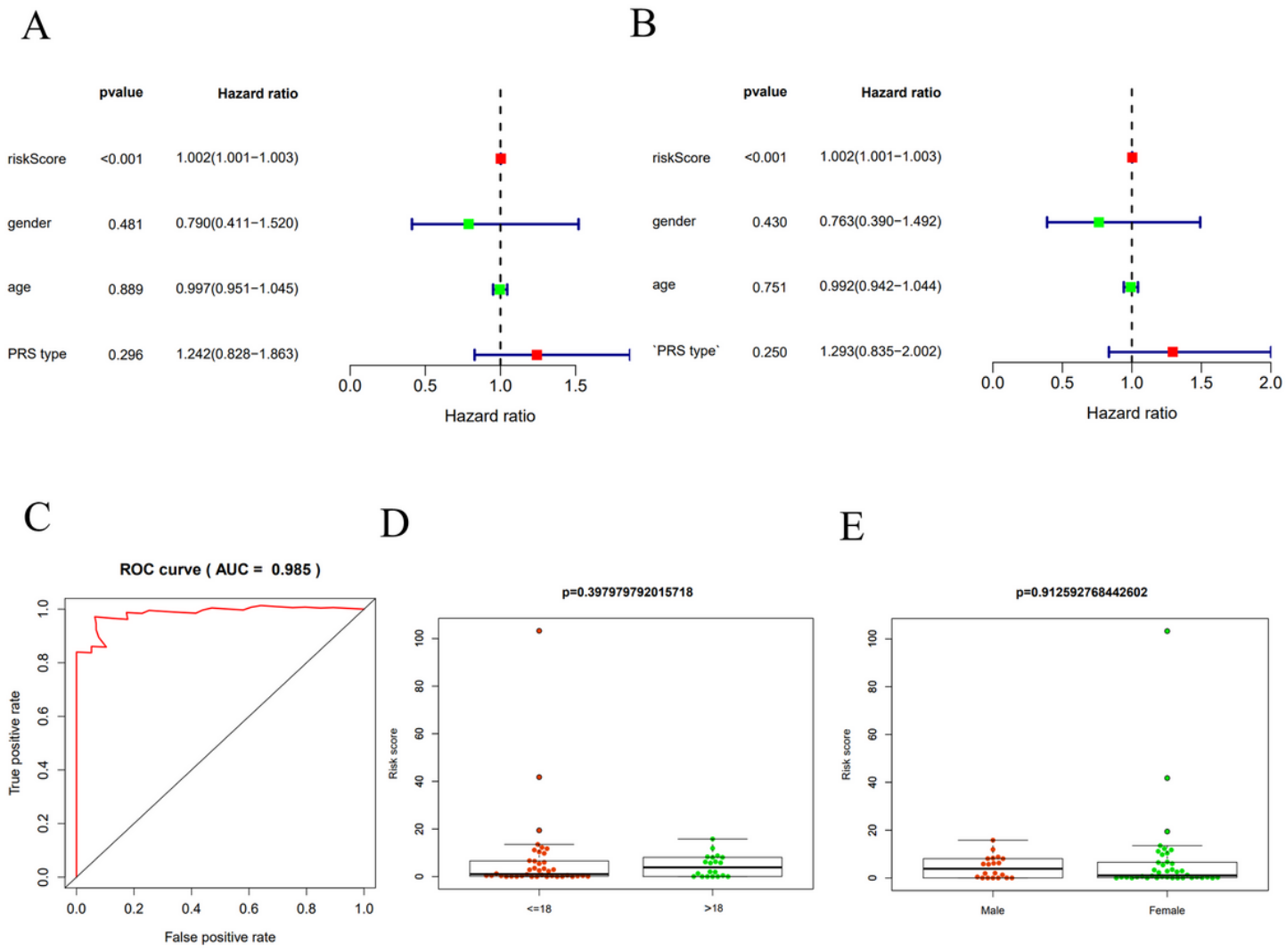

Figure 4

(A)The forest map of univariate Cox regression analysis and (B) multivariate Cox regression analysis in ES patients. (C)Receiver operating characteristic (ROC) curve validated the prognostic significance of the established prognostic model. (D) (E) Clinicopathological significance of the prognostic index of ES.

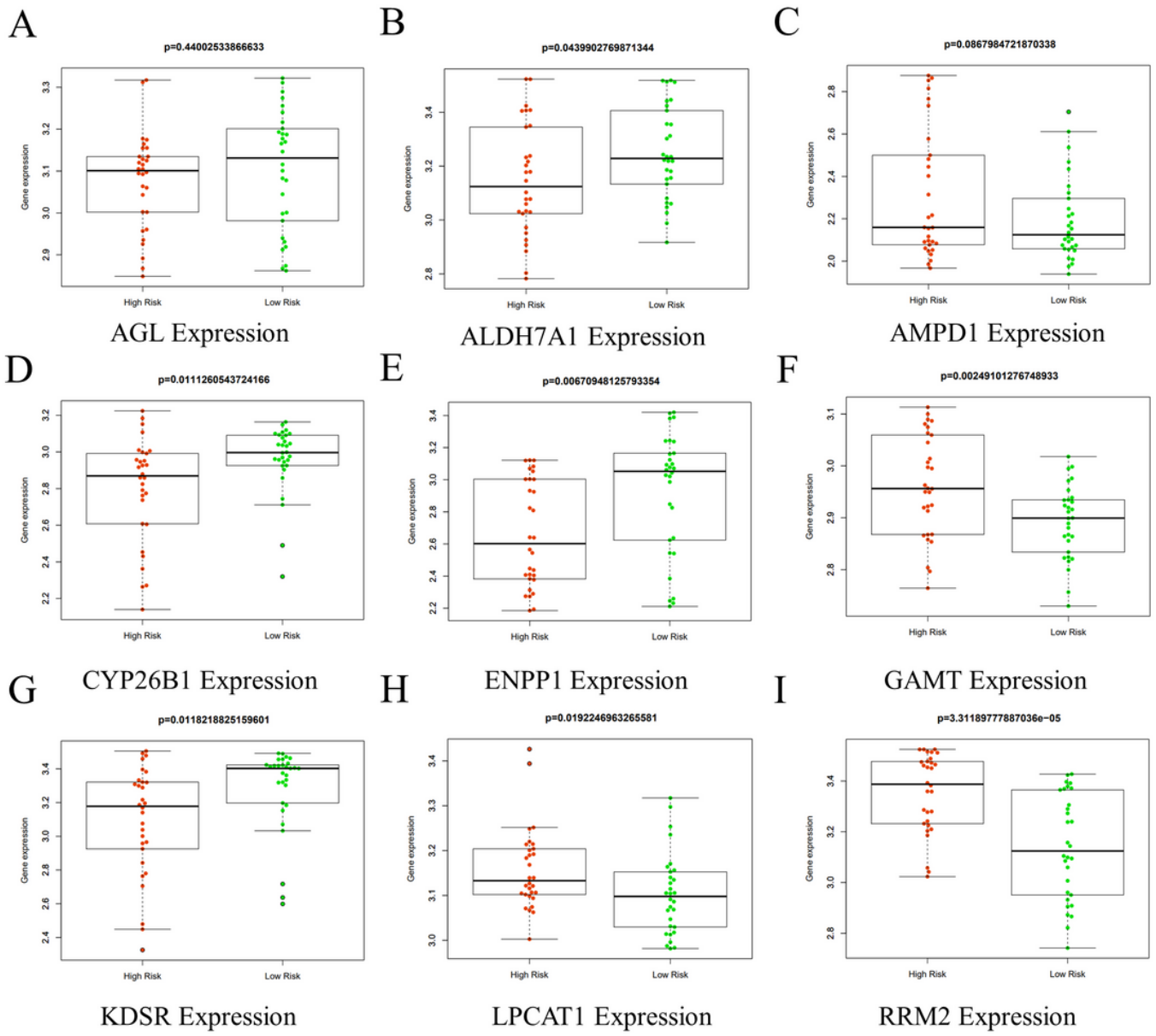

Figure 5

Genes of Metabolic prognostic index expressed in high RiskScore and low RiskScore groups