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Bioinformatics-based genetic analysis of correlation between esophageal cancer and iron death

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Abstract: Tounderstand whether there is a correlation between esophageal cancer and iron death, we performed a series of integrative analyses of gene expression profiles of esophageal cancer and driver genes of iron death through bioinformatics, and finally we identified 9 genes associated with iron death with significant overall prognostic variability of the genes. Through gene enrichment we understood that these 9 genes were associated with various pathways such as autophagy and metabolism. Among them, CDO1 and YAP1 genes have the greatest impact on esophageal cancer and may become potential prognostic markers for esophageal cancer.

1. Introduction

Esophageal cancer is one of the common malignant tumors in the gastrointestinal tract[1], which originates from the malignant transformation of esophageal mucosal epithelial basal cells, and the main pathological types are esophageal adenocarcinoma and esophageal squamous cell carcinoma[2]. The typical clinical symptom of esophageal cancer is progressive dysphagia, which is often manifested as choking sensation in the early stage of the disease, and with the development of the disease, it may become difficult to eat and drink, and even accompanied by persistent pain in the front and back of the chest and burning sensation behind the sternum [3]. Esophageal cancer is often associated with various complications after surgery, including laryngeal nerve injury, gastroesophageal reflux, anastomotic fistula, and incisional infection, and its prognosis is poor, with a five-year survival rate of only about 20% [4], making it one of the most common types of cancer occurring in Asia and Africa today [3]. The main risk factors are smoking, alcohol consumption, poor diet such as frequent consumption of hot and pickled foods, infections such as Helicobacter pylori and human papilloma virus, and family history [5]. The incidence of death from esophageal cancer is in the sixth place of global cancers, with a higher incidence in developing countries than in developed countries and a higher incidence in rural than in urban areas[6].

In 2012, the concept of iron death, a type of cell death due to iron-dependent lipid peroxidation and excessive production of reactive oxygen species, was first introduced. It is a novel form of programmed cell death that differs from other programmed cell death such as apoptosis and autophagy in that its mechanism of occurrence is related to iron overload, lipid peroxidation accumulation, and abnormal amino acid metabolism, and is biochemically characterized by the

accumulation of iron and reactive oxygen species, glutathione depletion, and lipid peroxidation [7]. Iron death, mainly through both transporter protein-dependent and enzyme-regulated pathways, plays an extremely critical role in the progression of several cancers, preventing the continued growth of malignant cells by mechanisms that encompass biochemical regulation of multiple genes, abnormal expression regulation with ROS metabolic communication signals, and interaction of Ras/Raf/MEK/ERK metabolic pathways. In this study, bioinformatics was used to identify iron death marker genes associated with esophageal cancer, and potential therapeutic targets were identified, providing a new direction for the future of esophageal cancer.

There is no bioinformatics-based exploration of the mechanisms associated with iron death driver genes in esophageal cancer, and this study provides new ideas for the diagnosis and treatment of esophageal cancer by analyzing public datasets and screening pivotal genes to explore the mechanisms of this novel apoptosis among esophageal cancer.

2. Materials and Methods

The Cancer Genome Atlas (TCGA) database was initiated jointly by the National Cancer and Oncology Institute and the National Human Genome Research Institute. Its database contains basic information on clinical cases and its gene-related information is relatively comprehensive, and the data includes both raw and standardized data. We first obtained the expression profiles, extracted the mapping information of GeneSymbol and ENSG_ID, mapped ENSG_ID to GeneSymbol, and took the median when there were multiple matches, and finally obtained the transformed expression profile information. Then, we obtained the clinical case information from it.The FerrDb database is a database of iron death regulators and disease relationships, from which we obtained iron death driver genes, and then filtered out genes that overlapped with the esophageal cancer gene profile to obtain 252 genes, and finally obtained 160 samples by collating clinical cases.

The String database covers more than 24 million proteins from more than 5,000 organisms, which is one of the largest protein interactions database covering the most species and the largest interactions information. The differentially expressed genes associated with iron death were uploaded and analyzed in String to predict the interaction relationships between proteins encoded by genes that may play an important role in esophageal carcinogenesis.

3. Results

We screened 160 samples and extracted 252 genes that overlapped with iron death, and finally identified 9 genes associated with iron death with significant prognostic variability in genes overall. We observed significant prognostic variability in the assessment of prognostic variability in samples older than 65 years, in samples 65 years and younger, and in samples of males, while the variability in samples of females was not significant. Through gene enrichment we understand that these 9 genes are associated with various pathways such as autophagy and metabolism. Among them, CDO1 and YAP1 genes have the greatest impact on esophageal cancer and may become potential prognostic markers for esophageal cancer.

3.1. Lasso and Analysis

In this study, we have screened 252 genes whose expression profiles of esophageal cancer overlap with iron death, and we used the R package glmnet, integrated survival time, survival status and gene expression data, and performed regression analysis using the lasso-cox method. In addition we set a 10-fold cross-validation to obtain the optimal model. We set the Lambda value to 0.105614463473812 and finally obtained 9 genes, namely ATF3, ATG5, CDO1, CIRBP,

GABARAPL2, MEG3, MIR135B, TBK1, YAP1, as shown in Figure 1.

Fig 1

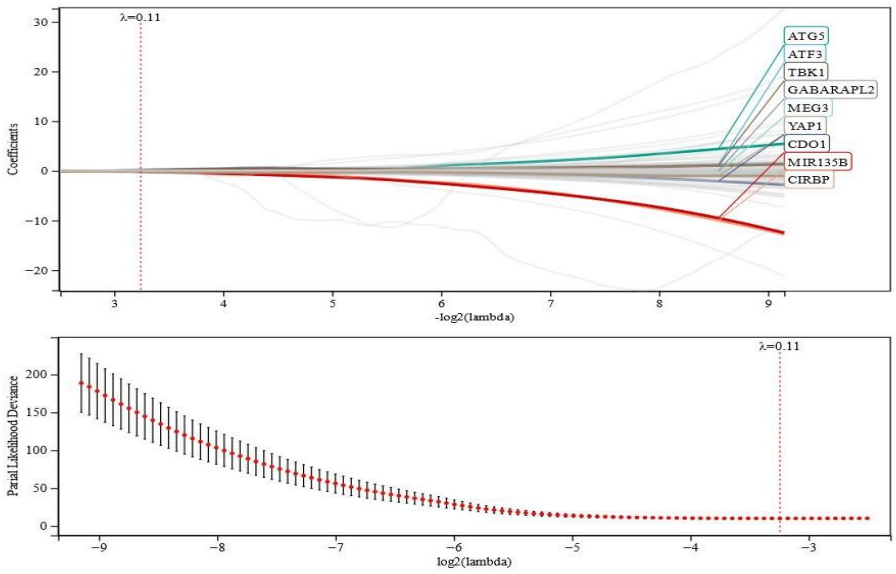
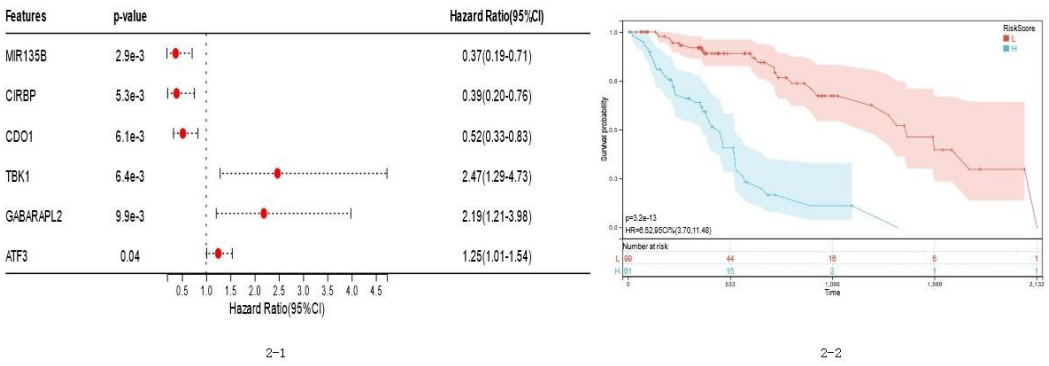


Figure 1: Lasso regression analysis graph.

3.2. Forest Plots and KM Survival Curves

Next, we assessed the prognostic significance of these features in 160 samples using the R package SURVIVAL, integrating data on survival time, survival status and 9 genes using the cox method. We found significant differences in their prognosis (Figure 2-1). Then, we calculated the optimal cut-off value of RiskScore using the R package maxstat, setting the minimum grouping sample size greater than 25% and the maximum sample size grouping less than 75%, and finally obtained the optimal cut-off value of 1.94867750415719, based on which the patients were divided into high and low groups, and further analyzed the prognosis of the two groups using the R package surv survfit function to analyze the prognostic difference between the two groups, and the significance of the prognostic difference between the samples of different groups was assessed using the logrank test method, and finally we observed a significant prognostic difference (Figure 2-2)

Fig 2



2-1 2-2

Figure 2: The forest plot is 2-1 and the KM survival curve is 2-2.

3.3. Prognostic Analysis of Individual Genes

To predict the effect of individual genes on the prognosis of esophageal cancer, we used the R package SURVIVAL, which integrates data on survival time, survival status, and nine genes, and assessed the prognostic significance of these gene characteristics in 160 samples using the cox method. The overall prognostic differential significance is shown in Figure 3: ATF3, MIR135, YAP1, and GABARAPL2 had more significant prognostic differentials relative to the other five genes.

Fig 3

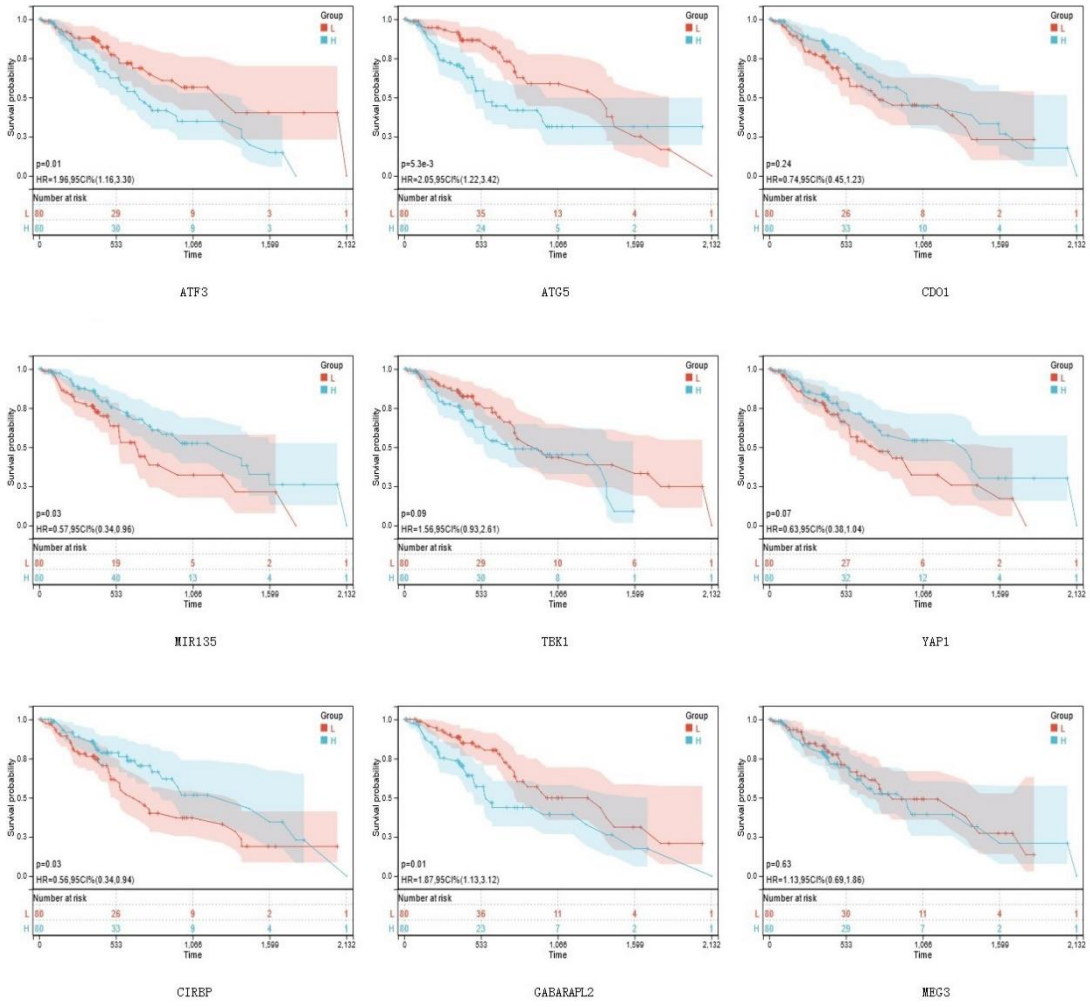


Figure 3: Survival curves for ATF3, ATG5, CDO1, MIR135B, TBK1, YAP1 ,CIRBP, GABARAPL2, MEG3in that order.

3.4. Prognostic Differences under the Influence of Gender and Age

Next, in order to understand the prognostic differences among the esophageal cancer samples due to age and gender, we ranked all samples according to the RiskStore in Lasso regression analysis from highest to lowest, and we considered the top 80 cases as high risk and the bottom 80 cases as low risk. Then we divided the sample into two groups according to the age of the sample, one group was over 65 years old and one group was 65 years old and below, and we performed prognostic difference analysis for each of the two groups to obtain the results in Figure 4. Then we divided all the samples

into two groups, male and female, and performed prognostic difference analysis for each of them to obtain the results in Figure 5. We could observe that there were significant prognostic differences. While in the female sample, the differences were not significant.

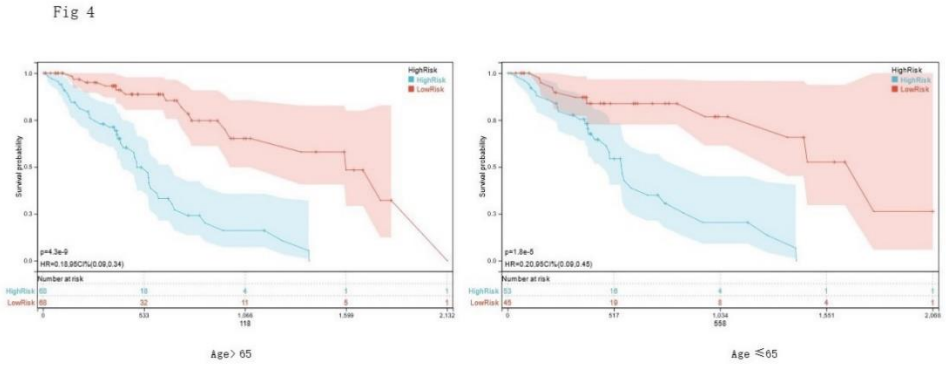


Figure 4: Survival curves using age 65 as a cut-off.

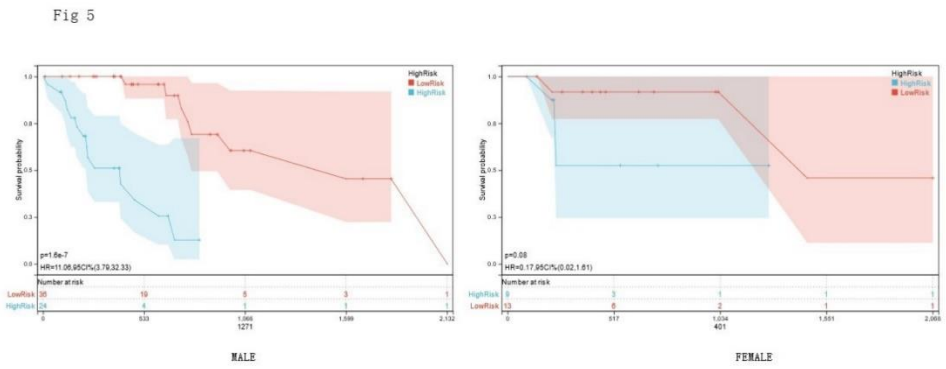


Figure 5: Survival curves by gender.

3.5. Gene Expression Analysis and ROC Analysis

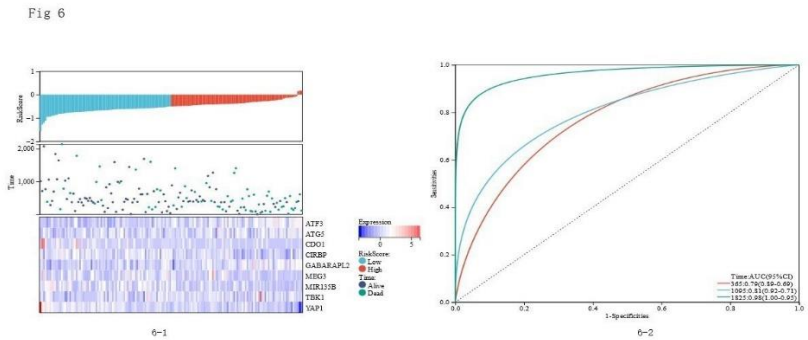


Figure 6: Gene expression analysis heat map for 6-1 and ROC analysis for 6-2.

We analyzed the relationship between different risk scores and patients' survival time, survival status and changes in the expression of each gene, and it can be observed that with the increase of risk scores, the survival rate of patients decreased significantly, and it can be known that among the nine genes screened, CDO1 and YAP1 genes have the greatest influence on esophageal cancer and are protective factors, and the expression shows a down-regulation trend with the increase of risk scores The rest of the genes were not significantly expressed (Figure 6-1). Next, we performed ROC

analysis using the R package pROC to obtain the AUC, specifically, we performed ROC analysis at 365, 1095, and 1825 time points using the survival time, survival status, and risk score of the patients, and evaluated the AUC using the ci function of pROC and confidence intervals to obtain the final AUC results (Figure 6-2).

3.6. Alignment Diagram

Using the R package rms, we integrated data on survival time, survival status, and 3 characteristics of age, sex, and risk score, and created a nomogram using the cox method to assess the prognostic significance of these characteristics in a sample of 160. The results are shown in Figure 7

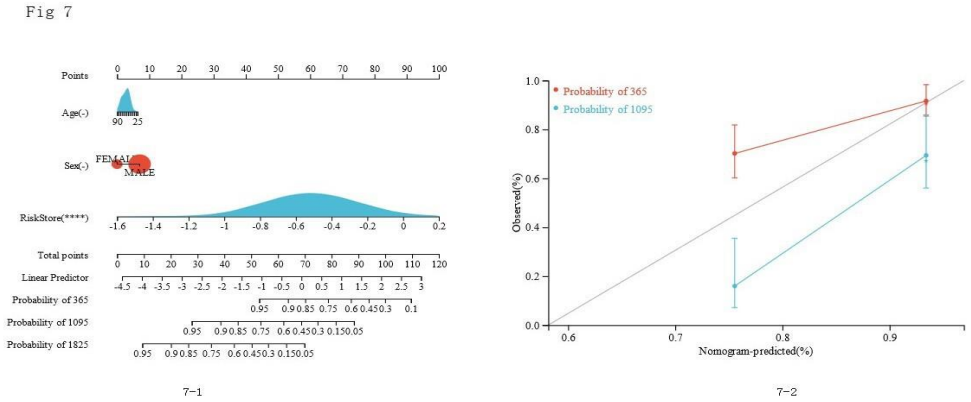


Figure 7: Nomogram is 7-1 and correction curve is 7-2.

3.7. Protein-protein Interaction Network and Gene Enrichment

We analyzed the protein-protein interaction network using 9 genes using STRING database, and we chose to add more proteins to observe in order to get more information to obtain Figure 8-1, where the gene with the highest interaction score was ATG5, and 3 of them did not form any network with other genes. The latest gene annotations of KEGG Pathway were obtained using the KEGG rest API as a background(8-2), and the genes were mapped to the background set for enrichment analysis using the R package clusterProfiler to obtain the results of gene set enrichment. The minimum gene set was set to 5, the maximum gene set to 5000, the P value of < 0.05 and a FDR of < 0.25.

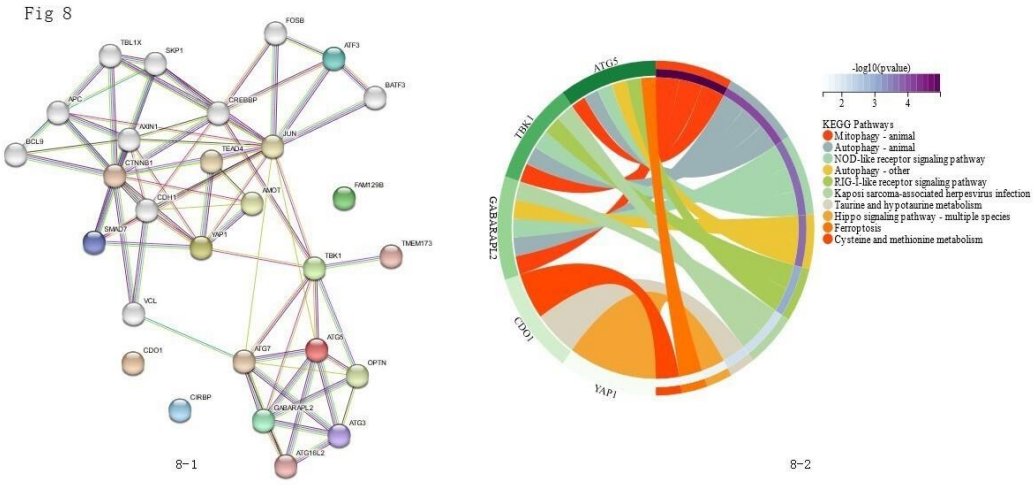


Figure 8: The protein-protein interaction diagram is 8-1 and the gene enrichment diagram is 8-2.

4. Discussion

Malignant neoplasm, as the second most common disease causing human death worldwide, has more than 18 million new cases and more than 9 million deaths each year[8]. The causes of tumor occurrence are not clear at present, but are generally believed to be the result of a combination of factors. In traditional Chinese medicine, it is believed that malignant tumors have the characteristics of pathological products such as qi stagnation, blood stasis, phlegm and so on, and the main triggering factors are external evil and toxicity, emotional and internal injury, and inappropriate diet. The etiology of esophageal cancer may be related to long-term poor dietary habits, smoking, alcohol consumption and other factors[9], and its incidence has obvious regional characteristics. According to the data released by the World Health Organization, the crude incidence rate and crude mortality rate of esophageal cancer in China ranked first in the world in 2020.

In this study, we first obtained the overlapping genes of esophageal cancer gene profile and iron death driver genes by analysis, and then obtained the differentially expressed genes related to iron death genes by screening, and found a total of 9 genes. Among them, ATF3 is a marker gene of iron death, indicating that iron death is related to the pathogenesis and prognosis of esophageal cancer, and in order to clarify the relationship between these genes and the pathogenesis and prognosis of esophageal cancer, we performed functional enrichment analysis and found that these genes are mainly involved in autophagy and metabolism, and also involved in NOD-like receptor signaling pathway and RIG-I-like receptor signaling pathway. NOD-like receptors are important components of the mammalian innate immune system, regulating immune and inflammatory responses, and are also known as NLRs, which induce the activation of cystein-1, thereby regulating the maturation of pro-inflammatory cytokines and triggering focal death. According to the results obtained in Figure 6-1, CDO1 and YAP1 are protective factors in the prognosis of esophageal cancer.CDO1 gene has been suggested as a potential tumor suppressor gene, and it was found that CDO1 is associated with the prognosis of prostate cancer patients and is also an independent prognostic factor in colon cancer [10].YAP1 is mainly associated with Hippo signaling pathway, and YAP1 has the ability to regulate metastatic invasion of cancer cells , and it also has pro-apoptotic and inhibitory effects, with both oncogene characteristics and tumor suppressor oncogene characteristics [10]. In our validation, we can see that as the expression of YAP1 decreases, the survival rate of patients decreases, therefore, among esophageal cancers, the YAP1 gene may have a pro-apoptotic role, and it, together with the CDO1 gene, could be a potential prognostic marker for esophageal cancer.

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