

Prognostic model of head and neck squamous cell carcinoma based on 12 ferroptosis-related genes

Sijia Li

The first affiliated hospital of China Medical University <https://orcid.org/0000-0001-6119-0440>

Hongyang Zhang

The First Affiliated Hospital of China Medical University

Wei Li (✉ wli@cmu.edu.cn)

The First Affiliated Hospital of China Medical University <https://orcid.org/0000-0001-9368-3397>

Research

Keywords: head and neck squamous cell carcinoma, ferroptosis, The Cancer Genome Atlas, prognosis

Posted Date: March 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-349666/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: The purpose of our study is establishing a model based on ferroptosis-related genes predicting the prognosis of patients with head and neck squamous cell carcinoma (HNSCC).

Methods: In our study, transcriptome and clinical data of HNSCC patients were from The Cancer Genome Atlas, ferroptosis-related genes and pathways were from Ferroptosis Signatures Database. Differentially expressed genes (DEGs) were screened by comparing tumor and adjacent normal tissues. Functional enrichment analysis of DEGs, protein-protein interaction network and gene mutation examination were applied. Univariate Cox regression analysis and least absolute shrinkage and selection operator (LASSO) regression were used to identified DEGs. The model was constructed by multivariate Cox regression analysis and verified by Kaplan-Meier analysis. The relationship between risk scores and other clinical features was also analyzed. Univariate and multivariate Cox analysis was used to verified the independence of our model. The model was evaluated by receiver operating characteristic analysis and calculation of the area under the curve (AUC). A nomogram model based on risk score, age, gender and TNM stages was constructed.

Results: We analyzed data including 500 tumor tissues and 44 adjacent normal tissues and 259 ferroptosis-related genes, then obtained 73 DEGs. Univariate Cox regression analysis screened out 16 genes related to overall survival, and LASSO analysis fingered out 12 of them with prognostic value. A risk score model based on these 12 genes was constructed by multivariate Cox regression analysis. According to the median risk score, patients were divided into high-risk group and low-risk group. The survival rate of high-risk group was significantly lower than that of low-risk group in Kaplan-Meier curve. Risk scores were related to T and grade. Univariate and multivariate Cox analysis showed our model was an independent prognostic factor. The AUC was 0.669. The nomogram showed high accuracy predicting the prognosis of HNSCC patients.

Conclusion: Our model based on 12 ferroptosis-related genes performed excellently in predicting the prognosis of HNSCC patients. Ferroptosis-related genes may be promising biomarkers for HNSCC treatment and prognosis.

Introduction

HNSCC is the sixth most common cancer worldwide and has been showing an increase in incidence in recent years[1]. In 2018, more than 500,000 cases of HNSCC were reported worldwide, with 380,000 yearly deaths[2]. HNSCC is a highly heterogeneous disease, with approximately 60 percents of patients being diagnosed in advanced stages[3]. These patients have a poor prognosis and no effective clinical treatments are currently available[4]. A major difficulty related to HNSCC is the lack of early diagnostic and predictive biomarkers[5]. Accordingly, biomarkers for HNSCC are urgently needed for an early identification, prognosis and treatment of this condition.

Ferroptosis is an iron-dependent and oxidative form of regulated cell death (RCD)[6]. It is induced through the production of reactive oxygen species (ROS) in cascade reactions of free unstable iron atoms with lipid peroxides[7]. An excessive accumulation of ROS is toxic and related to the damage of membrane structures and other cell-damaging reactions including oxidative stress, eventually leading to cell death[8]. Oxidative stress can induce DNA damage, impacts cellular signaling pathways and the development of tumor vessels, all of which contribute to the formation, proliferation and metastasis of cancers[9]. Promoting oxidative stress within cancer cells can lead to their death or apoptosis and, in this way, can be applied in the treatment of cancers[10]. Similarly, ferroptosis can be used in the treatment of cancers due to its capacity to activate oxidative stress. Many types of proteins are involved with processes related to the activation of ferroptosis[11], including iron metabolism and two key inhibitory processes[7]. One of these inhibitory processes involves a reduction in cystine transfer into cells by the cystine/glutamate antiporter system (Xc-)[12]. This process is related to the production of GSH[13], with low or absent levels of GSH inducing oxidative stress. The other inhibitory process involves a reduction in glutathione peroxidase 4 (GPX4)[6], which is related to lipid peroxidation[14]. Ferroptosis-related proteins play an important role in cancers and have been used as tumor markers[15]. As one example, GPX4 serves as a prognostic marker in patients with PDAC[16]. It has been suggested that other ferroptosis-related proteins may function as potential tumor markers.

Ferroptosis-related proteins have been shown to play an important role in HNSCC, in particular through their effects on GSH. As a key protein involved in ferroptosis, GSH levels are significantly altered in HNSCC and this imbalance in GSH may indicate a metastasis of HNSCC. Accordingly, changes in GSH levels have proved to serve as a prognostic factor for the diagnosis of HNSCC in its early stages[17]. Such results suggest that the genes regulating ferroptosis-related proteins can function as prognostic markers of HNSCC. As one example, SLC7A11 which impacts GSH by regulating cystine and affects amino acids in cancer cells, may provide a potential biomarker for HNSCC treatment[18]. Currently, research directed at constructing prognostic models for HNSCC have been based on single ferroptosis-related genes, such as the model based on CDGSH iron sulfur domain2 (CISD2)[19]. However, levels of the sensitivity and specificity associated with a single tumor marker are low. In contrast, multigene models have the potential of resolving these issues and therefore may prove more effective in identifying cancer stages and prognosis. The value of this approach has been revealed in predicting the prognosis of other cancers, especially in that of the prognosis model for HCC as based on ferroptosis-related genes[20]. Construction of a prognostic model for HNSCC as based on ferroptosis-related genes would be a decidedly warranted endeavor. Therefore, our goal in this study was to establish a prognostic model of HNSCC based on ferroptosis-related genes. Such a model would then afford a new and reliable prognostic marker for the treatment of HNSCC.

Materials & Methods

Data capturing

The RNA sequencing data for HNSCC patients including 500 tumor tissues and 44 adjacent normal tissues were downloaded from The Cancer Genome Atlas (TCGA; <https://cancergenome.nih.gov>) as well as corresponding clinical data. The data of ferroptosis-related genes was downloaded from the FerrDb (<http://www.zhounan.org/ferrdb/>).

The study need not be authorized by ethics committee, since it is of bioinformatics.

Ferroptosis-related DEGs analysis

The differential expressed genes (DEGs) were identified by the R package with the criteria of $|\log_2FC| > 0.5$ and false discovery rate (FDR) < 0.05 . Then, the expression levels of the DEGs in multiple samples were visualized by volcano plot and heatmap.

Functional enrichment analysis

Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed by "cluster profiler" R package for the functions of the DEGs. GO enrichment was carried out mainly from the following three levels: cellular components (CC), biological processes (BP), and molecular functions (MF), while KEGG analysis was about metabolic pathways and molecular mechanisms.

Construction of Protein-Protein Interaction (PPI) network

To further explore the relationship and interaction among ferroptosis-related DEGs, a PPI network was built by STRING (<https://string-db.org/>), and Cytoscape software (version 3.6.0) was used for an intuitive understanding of PPI.

Examination of gene mutations

For a further exploring of the connection between HNSCC and mutations of DEGs, gene mutation examination was needed. After downloading the exon data set of TCGA samples and extracting mutation data, 224 samples from TCGA was used to detect the mutation frequency of DEGs and analyze their translational effects. Waterfall chart was drawn to display the result. The analysis of gene mutation was based on Cancer Genomics Research Center (<http://www.cbioportal.org/>).

Signature establishment and verification

Univariate Cox regression analysis was used to identified the ferroptosis-related genes associated with overall survival (OS) by the "lrima" R package. The LASSO algorithm was used to identified the ferroptosis-related genes with prognostic values by the "glmnet" R package. The risk score was evaluated by the coefficient of each prognostic ferroptosis-related gene got from multivariate Cox regression analysis. The risk scoring formula was constructed as Risk scores = Risk score = $\beta_{\text{gene}(1)} \times \text{expression gene}(1) + \beta_{\text{gene}(2)} \times \text{expression gene}(2) + \dots + \beta_{\text{gene}(n)} \times \text{expression gene}(n)$. After patients were divided into high-risk and low-risk groups with the median risk score as the critical point, the Kaplan-Meier curve was performed to describe the relationship between survival probability and survival time for high-

risk group and low-risk group. The analysis including distribution of risk score, risk-related survival status and prognosis DEGs heatmap were also involved. Then the relationships among the risk score and clinical parameters were analyzed. Univariate Cox regression and multivariate Cox regression were used to identify the independent prognostic factors among risk score, age, gender, grade, stage and TNM. ROC analysis and calculated the area under the curve (AUC) were performed for evaluating the predictive performance of risk score.

Nomogram construction

To provide doctors and patients with a quantitative method for individual survival prediction, the prognostic nomogram model was constructed based on the regression algorithm combining age, gender and TNM stage with risk score. The consistency between the predicted results of the model and the actual results was appraised by calibration curves.

Statistical analysis

R language was applied in the TCGA database analysis. ROC curve and area under ROC curve were executed by "survival ROC" package in R software. R language (version 3.6.3) was used for all statistical analysis in this study. All statistical tests were bilateral tests, with statistical significance of $P < 0.05$.

Results

Identification ferroptosis-related DEGs in HNSCC

The RNA sequencing data and clinical data of HNSCC patients were obtained from TCGA and 259 ferroptosis-related genes were downloaded from FerrDb. 73 DEGs were identified between tumor tissues and normal tissues with 61 up-regulated genes and 12 down-regulated genes. (Figs,1A, 1B, 1C)

Functional enrichment of ferroptosis-related DEGs

GO and KEGG analyses were performed in 73 DEGs to gain a further research about their biological functions and pathway and the first 10 biological processes of them respectively were summarized. The related biological processes were mainly involved in response to oxidative stress, multicellular organismal homeostasis, cellular response to oxidative stress, carboxylic acid biosynthetic process, organic acid biosynthetic process, reactive oxygen species metabolic process, response to metal ion, peptidyl-serine phosphorylation, peptidyl-serine modification and superoxide anion generation. (Fig. 2A) The results of GO analysis about cell components and molecular function were also shown. (Fig. 2B,2C) In addition, the pathways in relation to DEGs were involved in HIF-1 signaling pathway, mTOR signaling pathway, AGE-RAGE signaling pathway in diabetic complications, cysteine and methionine metabolism, ferroptosis, necroptosis, fluid shear stress and atherosclerosis, microRNAs in cancer, central carbon metabolism in cancer, pancreatic cancer by KEGG analysis. (Fig. 2.D)

PPI network construction and crucial gene modules

The PPI network was constructed for a further exploring about the interactions among 73 DEGs. (Fig. 3A) There were 67 remaining DEGs after removing unconnected nodes. (Fig. 3B) The interactions among some basic genes were shown. (Fig. 3C)

Gene mutation analyses

The gene mutations of the genes were examined for the connection between HNSCC and mutations of 73 ferroptosis-related DEGs and nonsense mutation and missense mutation were the two most common types. There were 10 genes with mutation rate $\geq 5\%$, among which CDKN2A was the gene with the highest mutation rate. (Fig. 4)

Construction and assessment of the ferroptosis related DEGs prognostic signature

16 ferroptosis-related genes related to OS were chosen following the criteria of P value less than 0.05 by univariate Cox regression analysis. (Fig. 5) LASSO analysis was employed to shrink and choose prognosis-related ferroptosis genes to improve the accuracy. 12 genes involved prognosis were further identified from the 16 genes selected before by lambda method to construct the prognostic signature of HNSCC. (Fig. 6A, B). The risk score was calculated based on their expression level and associated Cox regression coefficient based on multivariate Cox regression.

Risk score = (0.2989744 x expression CISD2) + (-0.0013736 x expression EGFR) + (0.0553490 x expression ASNS) + (0.1433406 x expression TRIB3) + (0.0782387 x expression AURKA) + (0.1629319 x expression G6PD) + (0.1721795 x expression PRKAA2) + (-0.1792301 x expression SOCS1) + (0.0985754 x expression CAV1) + (0.0551493 x expression SLC7A5) + (-0.0726862 x expression CDKN2A) + (0.0939850 x expression DDIT4)

The patients with HNSCC were divided into high-risk and low-risk groups based on median risk score. In order to predict the clinical outcome of HNSC patients, the distribution of different risk groups (Fig. 7A) and the survival rate of patients (Fig. 7B) were defined according to the risk scores. The levels of expression of 12 ferroptosis-related genes in both high-risk and low-risk groups were shown in heatmap (Fig. 7C). It was shown by the Kaplan-Meier log-rank test that patients in the high-risk group had a worse OS than those in the low-risk group (Fig. 7D).

Subsequently, the relationships among risk scores and other clinical parameters for the clinical significance of risk scores in HNSCC were analyzed. As a result, increasing risk scores were associated with higher T stages (P = 0.007) (Fig. 8A) and progressive tumor grades (P = 0.023) (Fig. 8B).

It was confirmed by univariate (Fig. 9A) and multivariate (Fig. 9B) COX regression analysis that the risk score was an independent prognostic predictor for the OS of patients with HNSCC (HR = 1.071, P < 0.001). ROC analysis was used to evaluate the predictive performance of the risk score, and the AUC of risk score was 0.669 (Fig. 9C), which was higher than that of other risk factors such as gender, age, and TNM stage.

Constructing a predictive nomogram

After statistical analysis, five independent clinical variables were chosen to combine with risk scores to build a comprehensive model for monitoring progression in HNSCC, including age, gender, T stages, M stages and N stages. According to LASSO logistic regression algorithm, it was shown in our nomogram that the survival rate of HNSCC individually was constructed based on these five clinical indicators and risk scores. (Fig. 10A) Moreover, the nomogram was used to predict the 1-year, 3-year and 5-year OS of patients (n = 447). The calibration curves were used to describe the good curve fit between 1-year, 3-year and 5-year progress events and actual observations. (Fig. 10B, C, D)

Discussion

The results of our study provide the first evidence demonstrating the feasibility of combining ferroptosis-related genes in a multigene model to predict the prognosis of HNSCC.

To accomplish this goal, 73 DEGs were obtained by identifying differentially expressed ferroptosis-related genes between tumor and normal tissues. To further verify the potential role of ferroptosis-related DEGs, GO and KEGG enrichment analyses were performed. Results of these analyses showed that these genes were abundantly expressed in processes involving oxidative stress, superoxide anion generation, reactive oxygen species metabolic processes, cellular responses to oxidative stress and peptidyl-serine phosphorylation, thus indicating that these 73 DEGs may play an important role in HNSCC. Oxidative stress is induced by excess formation of ROS, including superoxide anion; and, the generation of superoxide anions and ROS metabolic processes are associated with the proliferation, differentiation and growth of HNSCC[21, 22]. In addition, oxidative stress, which is associated with smoking[23] and HPV infection[24], also represents a source for HNSCC[25, 26]. Peptidyl-serine phosphorylation, like Akt phosphorylation, is sensitive to oxidative stress by inactivating PRAS40 and removing mTORC1[27]; and, Akt phosphorylation is an indispensable step in the carcinogenesis of HNSCC[28]. This Akt phosphorylation is closely related to the disease progression of HNSCC[29] as can be achieved with many downstream targets of key cellular processes like GSK-3 β , CREB1 and TSC2[30].

Among these 73 DEGs, 12 genes with prognostic value were screened to establish the risk scoring model. Several proteins with increased expression levels were accompanied with poor prognosis, such as SLC7A5, EGFR, CISD2 and ASNS. Levels of SLC7A5 were highly expressed in HNSCC[31] and are closely related to tumor size and grade, effects which constitute the Xc system in ferroptosis[32] and provide amino acids for promotion of cancer cell growth[33]. EGFR, which affects GSH through the phosphorylation of Akt[34], is related to the later stages, tumor size, invasion, decreased survival rates and poor prognosis of HNSCC[35, 36]. Further evidence indicating a role for EGFR has been demonstrated in response to treatment with Cetuximab, an EGFR-targeted drug shown to have survival benefits for metastatic HNSCC disease[4]. CISD2 encodes the NEET protein[37], NAF-1, which is related to tumor growth and metastasis by the iron ion[38]. CISD2 can be used as a molecular biomarker indicating a poor prognosis of HNSCC[19] due to its significant relationships with T stage, lymph node metastasis, clinical

stage and disease progression of HNSCC. ASNS affects GSH synthesis and participates in tumor growth, with the protein related to ASNS being associated with metastasis and recurrence of HNSCC[39, 40]. Based on results from previous studies, these 12 ferroptosis-related genes are closely related to the development and treatment of HNSCC and may thus serve as reliable markers in identifying potential therapeutic targets for HNSCC patients.

Based on our multigene model, significant differences in risk scores of head and neck cancer patients are present among different T-stage groups. These results suggests that our model correlates with T staging and can function as a reliable indicator in predicting tumor malignancy. Furthermore, our model has a better ability of predicting prognosis for HNSCC than that of TNM staging, with increased AUC (Area Under Curve) values being obtained with ROC analysis. In addition, our model shows a better prognostic capacity based on AUC values as compared with previously published models based on TCGA HNSCC database analysis[41–43]. The enhanced ability of our model in evaluating the prognosis of HNSCC should substantially improve the clinical treatment of this condition.

Two notable limitations present in our study include: 1) the retrospective nature of the study, which will require data from prospective studies to verify the prognostic ability of the model and substantiate our findings and 2) the bioinformatics analysis performed fails to reveal potential functional mechanisms that may be involved with this model.

Conclusions

In a word, the prediction model we built for the prognosis of HNSCC patients, is based on 12 ferroptosis-related genes and clinical characters of patients. And it can provide a reliable prediction about OS to patients with HNSCC. It provides new markers for the treatment of HNSCC.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Data availability could be obtained from the TCGA website.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the educational commission of Liaoning Province in China (Numbers: QN2019009).

Author contribution statement:

Sijia Li analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Hongyang Zhang prepared the figures and/or tables and approved the final draft.

Wei Li conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Acknowledgment

None.

Authors' information

¹Department of Otolaryngology, The first affiliated hospital of China Medical University, Shenyang, Liaoning, China

References

1. Haddad RI, Shin DM: **Recent advances in head and neck cancer.** *N Engl J Med* 2008, **359**(11):1143-1154.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.** *CA Cancer J Clin* 2018, **68**(6):394-424.
3. Marur S, Forastiere AA: **Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment.** *Mayo Clin Proc* 2016, **91**(3):386-396.
4. Winkquist E, Agbassi C, Meyers BM, Yoo J, Chan KKW, Head, Neck Disease Site G: **Systemic therapy in the curative treatment of head and neck squamous cell cancer: a systematic review.** *J Otolaryngol Head Neck Surg* 2017, **46**(1):29.
5. Shen Y, Liu J, Zhang L, Dong S, Zhang J, Liu Y, Zhou H, Dong W: **Identification of Potential Biomarkers and Survival Analysis for Head and Neck Squamous Cell Carcinoma Using Bioinformatics Strategy: A Study Based on TCGA and GEO Datasets.** *Biomed Res Int* 2019, **2019**:7376034.
6. Latunde-Dada GO: **Ferroptosis: Role of lipid peroxidation, iron and ferritinophagy.** *Biochim Biophys Acta Gen Subj* 2017, **1861**(8):1893-1900.

7. Skoupilova H, Michalova E, Hrstka R: **Ferroptosis as a New Type of Cell Death and its Role in Cancer Treatment.** *Klin Onkol* 2018, **31**(Suppl 2):21-26.
8. Kajarabille N, Latunde-Dada GO: **Programmed Cell-Death by Ferroptosis: Antioxidants as Mitigators.** *Int J Mol Sci* 2019, **20**(19).
9. Saha SK, Lee SB, Won J, Choi HY, Kim K, Yang GM, Dayem AA, Cho SG: **Correlation between Oxidative Stress, Nutrition, and Cancer Initiation.** *Int J Mol Sci* 2017, **18**(7).
10. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A: **Oxidative Stress: Harms and Benefits for Human Health.** *Oxid Med Cell Longev* 2017, **2017**:8416763.
11. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R, Tang D: **Ferroptosis: process and function.** *Cell Death Differ* 2016, **23**(3):369-379.
12. Xu T, Ding W, Ji X, Ao X, Liu Y, Yu W, Wang J: **Molecular mechanisms of ferroptosis and its role in cancer therapy.** *J Cell Mol Med* 2019, **23**(8):4900-4912.
13. Sato H, Tamba M, Kuriyama-Matsumura K, Okuno S, Bannai S: **Molecular cloning and expression of human xCT, the light chain of amino acid transport system xc.** *Antioxid Redox Signal* 2000, **2**(4):665-671.
14. Imai H, Matsuoka M, Kumagai T, Sakamoto T, Koumura T: **Lipid Peroxidation-Dependent Cell Death Regulated by GPx4 and Ferroptosis.** *Curr Top Microbiol Immunol* 2017, **403**:143-170.
15. Xia X, Fan X, Zhao M, Zhu P: **The Relationship between Ferroptosis and Tumors: A Novel Landscape for Therapeutic Approach.** *Curr Gene Ther* 2019, **19**(2):117-124.
16. Dai E, Han L, Liu J, Xie Y, Zeh HJ, Kang R, Bai L, Tang D: **Ferroptotic damage promotes pancreatic tumorigenesis through a TMEM173/STING-dependent DNA sensor pathway.** *Nat Commun* 2020, **11**(1):6339.
17. Dequanter D, Dok R, Nuyts S: **Basal oxidative stress ratio of head and neck squamous cell carcinomas correlates with nodal metastatic spread in patients under therapy.** *Onco Targets Ther* 2017, **10**:259-263.
18. Hemon A, Louandre C, Lailier C, Godin C, Bottelin M, Morel V, Francois C, Galmiche A, Saidak Z: **SLC7A11 as a biomarker and therapeutic target in HPV-positive head and neck Squamous Cell Carcinoma.** *Biochem Biophys Res Commun* 2020, **533**(4):1083-1087.
19. Yang L, Hong S, Wang Y, He Z, Liang S, Chen H, He S, Wu S, Song L, Chen Y: **A novel prognostic score model incorporating CDGSH iron sulfur domain2 (CISD2) predicts risk of disease progression in laryngeal squamous cell carcinoma.** *Oncotarget* 2016, **7**(16):22720-22732.
20. Liang JY, Wang DS, Lin HC, Chen XX, Yang H, Zheng Y, Li YH: **A Novel Ferroptosis-related Gene Signature for Overall Survival Prediction in Patients with Hepatocellular Carcinoma.** *Int J Biol Sci* 2020, **16**(13):2430-2441.
21. Diwanji N, Bergmann A: **An unexpected friend - ROS in apoptosis-induced compensatory proliferation: Implications for regeneration and cancer.** *Semin Cell Dev Biol* 2018, **80**:74-82.

22. Panieri E, Santoro MM: **ROS homeostasis and metabolism: a dangerous liason in cancer cells.** *Cell Death Dis* 2016, **7**(6):e2253.
23. Golbidi S, Li H, Laher I: **Oxidative Stress: A Unifying Mechanism for Cell Damage Induced by Noise, (Water-Pipe) Smoking, and Emotional Stress-Therapeutic Strategies Targeting Redox Imbalance.** *Antioxid Redox Signal* 2018, **28**(9):741-759.
24. Georgescu SR, Mitran CI, Mitran MI, Caruntu C, Sarbu MI, Matei C, Nicolae I, Tocut SM, Popa MI, Tampa M: **New Insights in the Pathogenesis of HPV Infection and the Associated Carcinogenic Processes: The Role of Chronic Inflammation and Oxidative Stress.** *J Immunol Res* 2018, **2018**:5315816.
25. Sabatini ME, Chiocca S: **Human papillomavirus as a driver of head and neck cancers.** *Br J Cancer* 2020, **122**(3):306-314.
26. von Witzleben A, Wang C, Laban S, Savelyeva N, Ottensmeier CH: **HNSCC: Tumour Antigens and Their Targeting by Immunotherapy.** *Cells* 2020, **9**(9).
27. Chamcheu JC, Roy T, Uddin MB, Banang-Mbeumi S, Chamcheu RN, Walker AL, Liu YY, Huang S: **Role and Therapeutic Targeting of the PI3K/Akt/mTOR Signaling Pathway in Skin Cancer: A Review of Current Status and Future Trends on Natural and Synthetic Agents Therapy.** *Cells* 2019, **8**(8).
28. Amornphimoltham P, Sriuranpong V, Patel V, Benavides F, Conti CJ, Sauk J, Sausville EA, Molinolo AA, Gutkind JS: **Persistent activation of the Akt pathway in head and neck squamous cell carcinoma: a potential target for UCN-01.** *Clin Cancer Res* 2004, **10**(12 Pt 1):4029-4037.
29. Li Z, Yang Z, Passaniti A, Lapidus RG, Liu X, Cullen KJ, Dan HC: **A positive feedback loop involving EGFR/Akt/mTORC1 and IKK/NF- κ B regulates head and neck squamous cell carcinoma proliferation.** *Oncotarget* 2016, **7**(22):31892-31906.
30. Mundi PS, Sachdev J, McCourt C, Kalinsky K: **AKT in cancer: new molecular insights and advances in drug development.** *Br J Clin Pharmacol* 2016, **82**(4):943-956.
31. Zhang Z, Liu R, Shuai Y, Huang Y, Jin R, Wang X, Luo J: **ASCT2 (SLC1A5)-dependent glutamine uptake is involved in the progression of head and neck squamous cell carcinoma.** *Br J Cancer* 2020, **122**(1):82-93.
32. Yoshida GJ: **The Harmonious Interplay of Amino Acid and Monocarboxylate Transporters Induces the Robustness of Cancer Cells.** *Metabolites* 2021, **11**(1).
33. Lu X: **The Role of Large Neutral Amino Acid Transporter (LAT1) in Cancer.** *Curr Cancer Drug Targets* 2019, **19**(11):863-876.
34. Silva-Oliveira RJ, Melendez M, Martinho O, Zanon MF, de Souza Viana L, Carvalho AL, Reis RM: **AKT can modulate the in vitro response of HNSCC cells to irreversible EGFR inhibitors.** *Oncotarget* 2017, **8**(32):53288-53301.
35. Freudlsperger C, Burnett JR, Friedman JA, Kannabiran VR, Chen Z, Van Waes C: **EGFR-PI3K-AKT-mTOR signaling in head and neck squamous cell carcinomas: attractive targets for molecular-oriented therapy.** *Expert Opin Ther Targets* 2011, **15**(1):63-74.

36. Chu PY, Tai YL, Shen TL: **Grb7, a Critical Mediator of EGFR/ErbB Signaling, in Cancer Development and as a Potential Therapeutic Target.** *Cells* 2019, **8**(5).
37. Karmi O, Marjault HB, Pesce L, Carloni P, Onuchic JN, Jennings PA, Mittler R, Nechushtai R: **The unique fold and lability of the [2Fe-2S] clusters of NEET proteins mediate their key functions in health and disease.** *J Biol Inorg Chem* 2018, **23**(4):599-612.
38. Mittler R, Darash-Yahana M, Sohn YS, Bai F, Song L, Cabantchik IZ, Jennings PA, Onuchic JN, Nechushtai R: **NEET Proteins: A New Link Between Iron Metabolism, Reactive Oxygen Species, and Cancer.** *Antioxid Redox Signal* 2019, **30**(8):1083-1095.
39. Balasubramanian MN, Butterworth EA, Kilberg MS: **Asparagine synthetase: regulation by cell stress and involvement in tumor biology.** *Am J Physiol Endocrinol Metab* 2013, **304**(8):E789-799.
40. Ghias K, Rehmani SS, Razzak SA, Madhani S, Azim MK, Ahmed R, Khan MJ: **Mutational landscape of head and neck squamous cell carcinomas in a South Asian population.** *Genet Mol Biol* 2019, **4242**(3):526-542.
41. Li C, Wu ZH, Yuan K: **Autophagy-Related Signature for Head and Neck Squamous Cell Carcinoma.** *Dis Markers* 2020, **2020**:8899337.
42. Xu X, Li M, Hu J, Chen Z, Yu J, Dong Y, Sun C, Han J: **Expression profile analysis identifies a two-gene signature for prediction of head and neck squamous cell carcinoma patient survival.** *J Cancer Res Ther* 2018, **14**(7):1525-1534.
43. Wu S, Dai X, Xie D: **Identification and Validation of an Immune-Related RNA Signature to Predict Survival of Patients With Head and Neck Squamous Cell Carcinoma.** *Front Genet* 2019, **10**:1252.

Figures

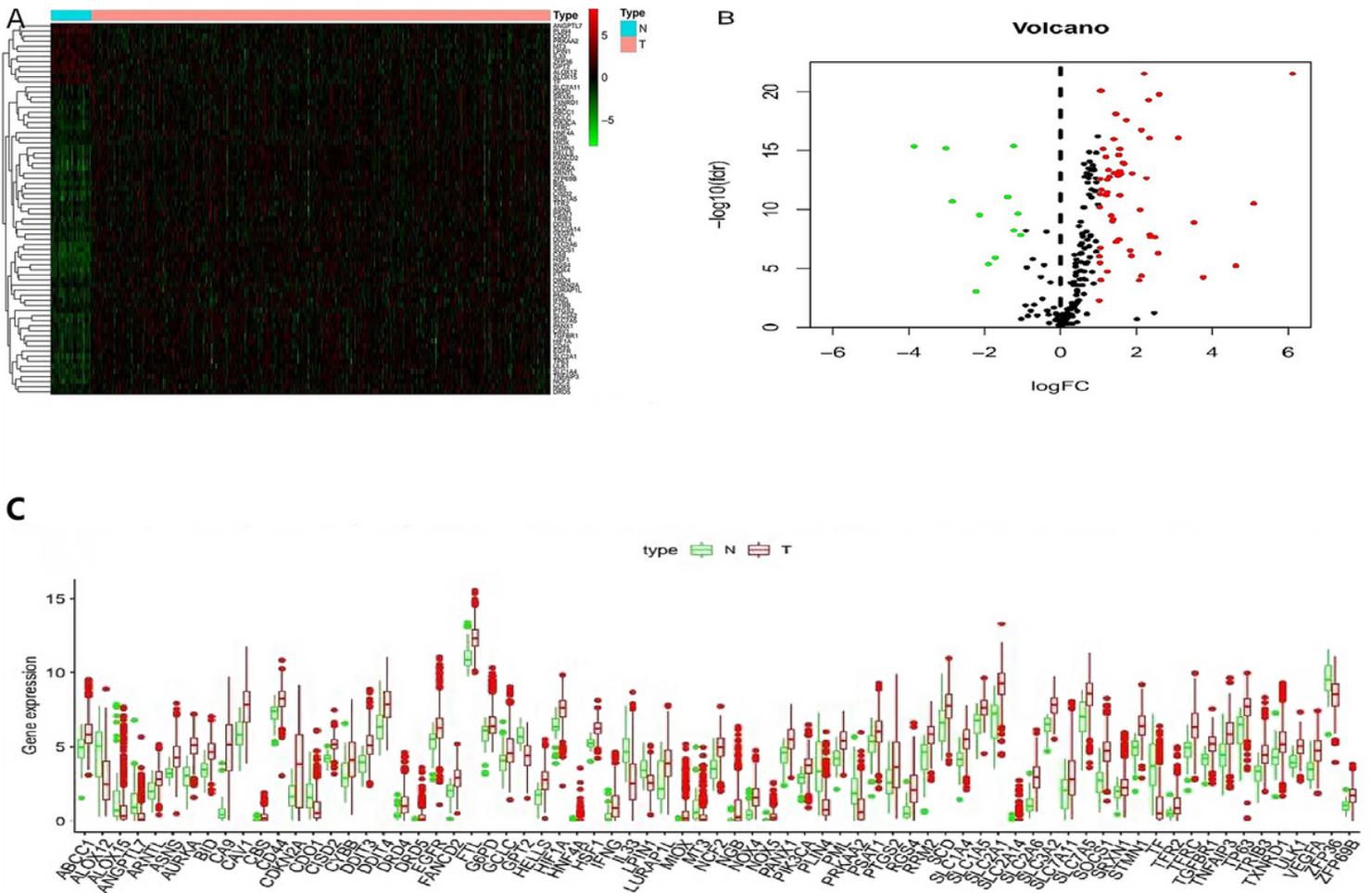


Figure 1

Identification of the ferroptosis-related DEGs between HNSCC and adjacent normal tissues. (A) The heatmap showed the expression of 73 DEGs in HNSCC tissues and adjacent normal tissues. The X-axis stood for the samples and the Y-axis represented DEGs. Up-regulated genes were shown in red, down-regulated genes were shown in green and non-differential genes were in black. (B) The volcano map showed that there were 61 up-regulated genes and 12 down-regulated genes. (C) The expression of 73 DEGs was also showed in the box plot, in which each red box stood for a gene in HNSCC tissues and each green one represented a gene in normal tissues.

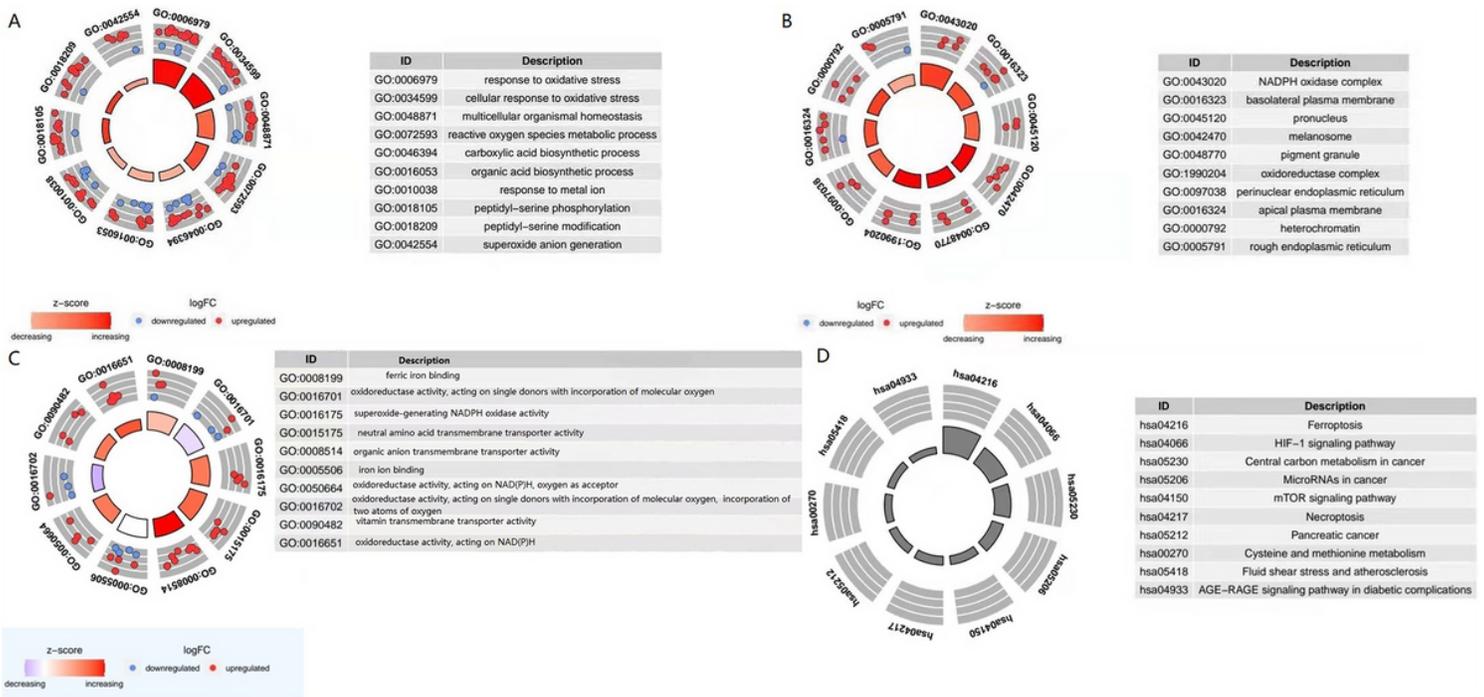


Figure 2

Functional enrichment analysis. (A-C) GO analysis showed the pathways involved in DEGs, including cellular components (CC), biological processes (BP), and molecular functions (MF). (D) KEGG analysis showed signaling pathways associated with DEGs.

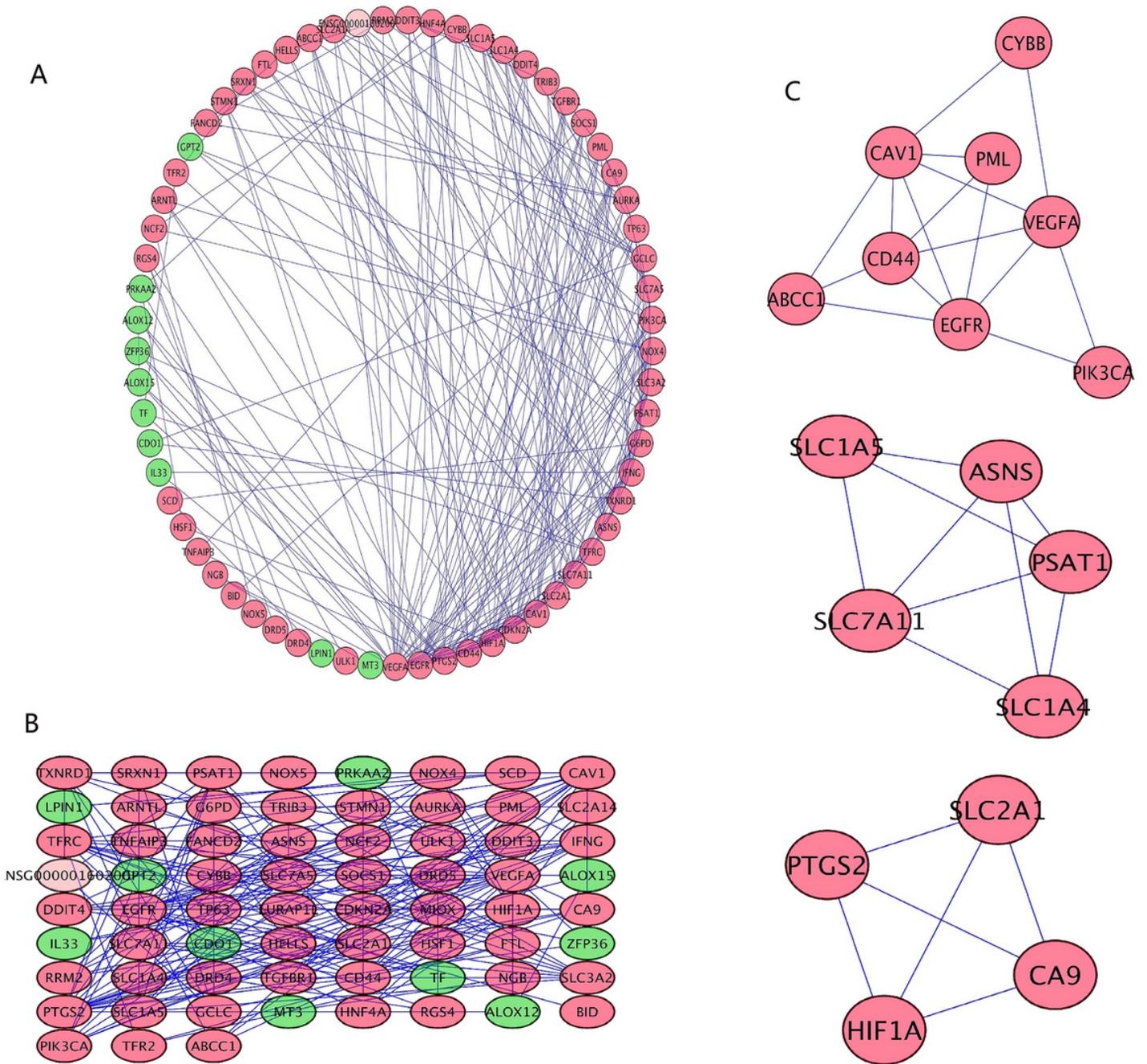


Figure 3

Construction of protein-protein interaction network. (A) The PPI network was built for the further study about the interactions among 73 DEGs. (B) After removing unconnected nodes, there were 67 DEGs. (C) Three gene modules were shown.

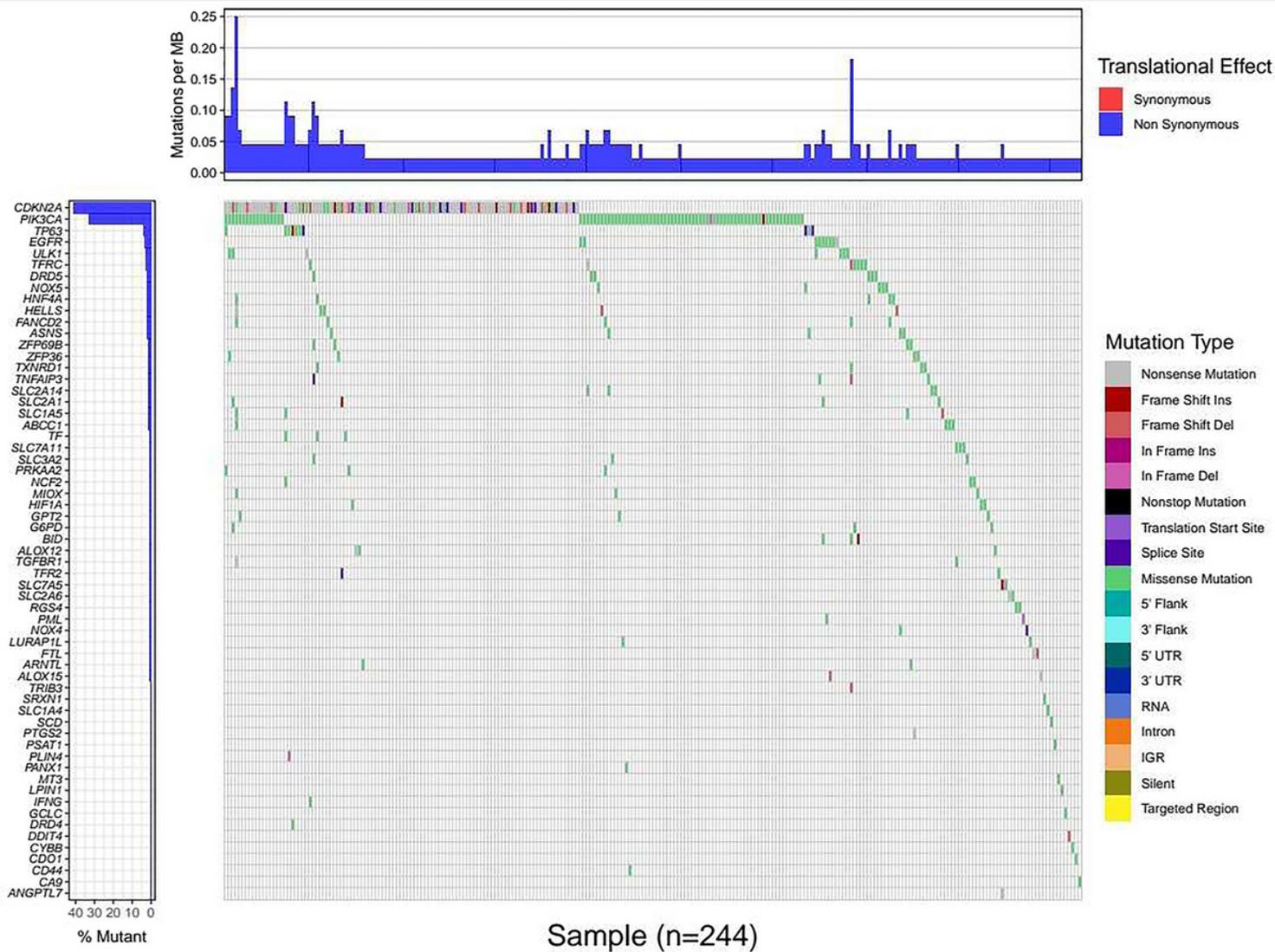


Figure 4

Gene mutation analyses. A total of 10 genes had the mutation rate more than 5%, and CDKN2A had the highest mutation rate in DEGs, which was 41%.

	pvalue	Hazard ratio
CISD2	0.031	1.347(1.028–1.766)
EGFR	0.029	1.120(1.012–1.239)
ASNS	0.003	1.207(1.064–1.369)
TRIB3	<0.001	1.308(1.136–1.506)
SLC3A2	0.012	1.245(1.049–1.478)
AURKA	0.019	1.216(1.032–1.433)
HSF1	0.041	1.309(1.011–1.694)
G6PD	0.015	1.159(1.030–1.305)
PRKAA2	0.031	1.147(1.013–1.299)
SOCS1	0.040	0.877(0.773–0.994)
CAV1	0.019	1.115(1.018–1.222)
SLC7A11	0.039	1.082(1.004–1.165)
SLC7A5	0.011	1.159(1.035–1.298)
CDKN2A	0.010	0.932(0.883–0.983)
TXNRD1	0.009	1.130(1.031–1.238)
DDIT4	0.029	1.131(1.013–1.263)

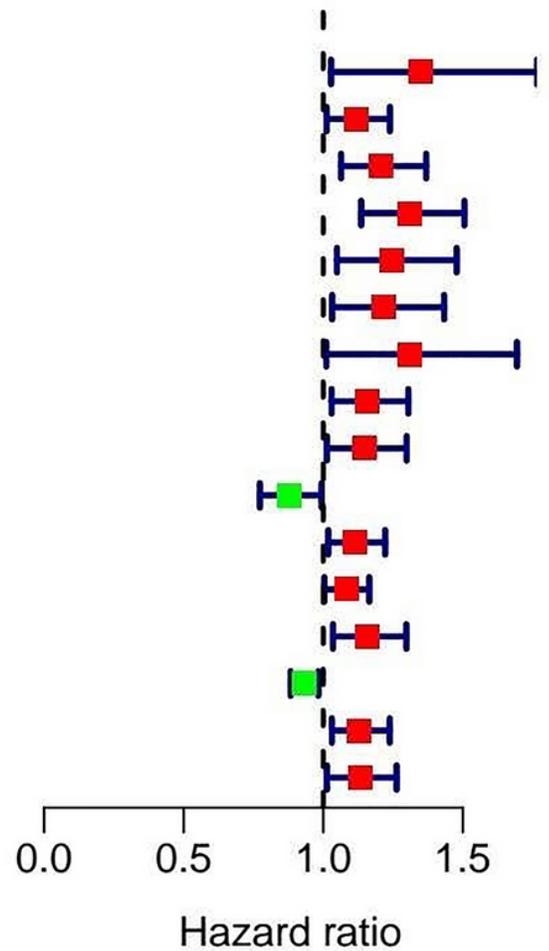


Figure 5

Identification of DEGs about overall survival rate. There were 16 DEGs related to OS in total in the forest map by the univariate Cox regression. Up-regulated genes were red and down-regulated genes were green.

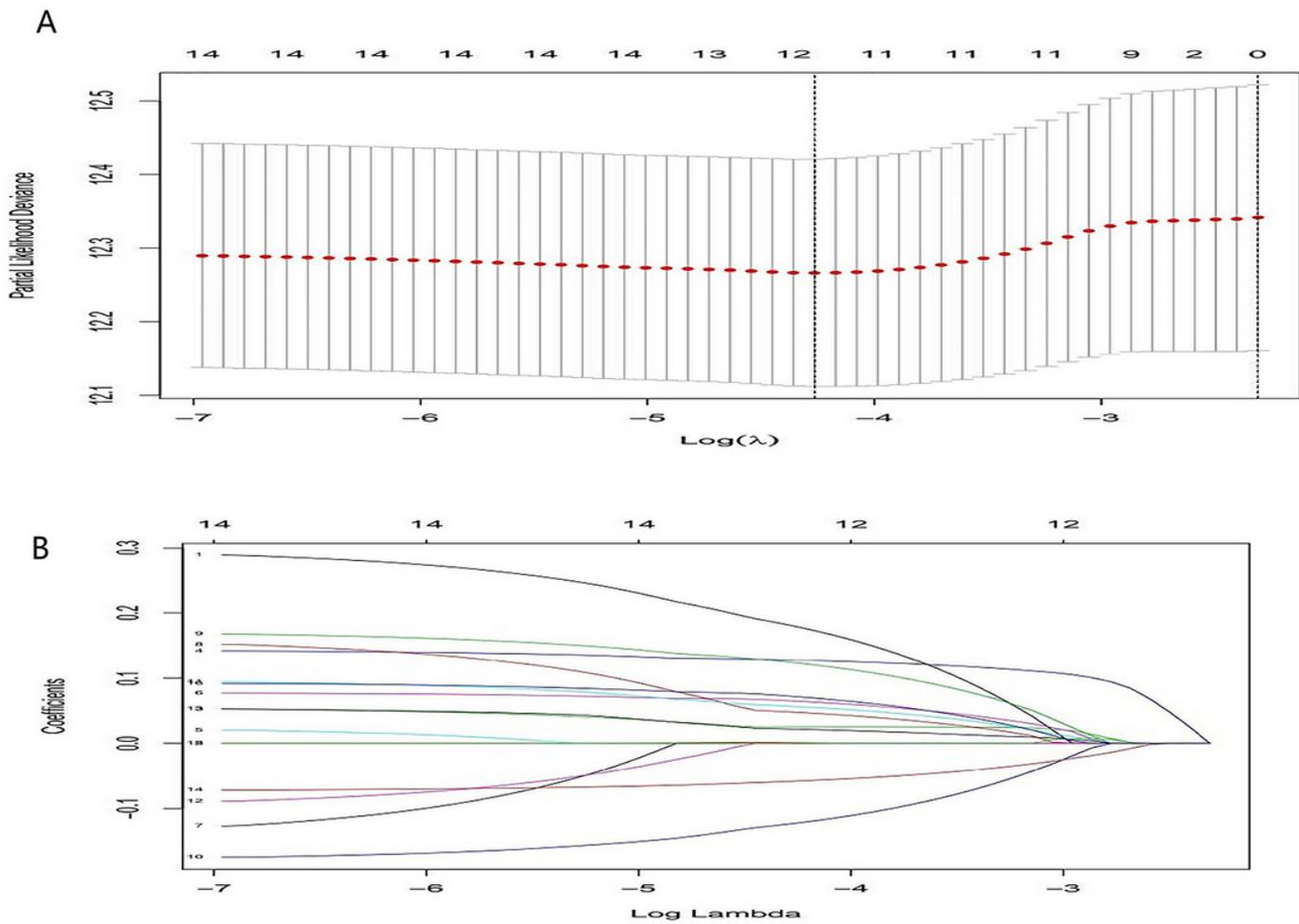


Figure 6

Selection for ferroptosis-related genes of prognostic value. (A–B) 12 ferroptosis-related genes associated with the prognosis of HNSCC were identified in TCGA training cohort by the LASSO Cox regression analysis.

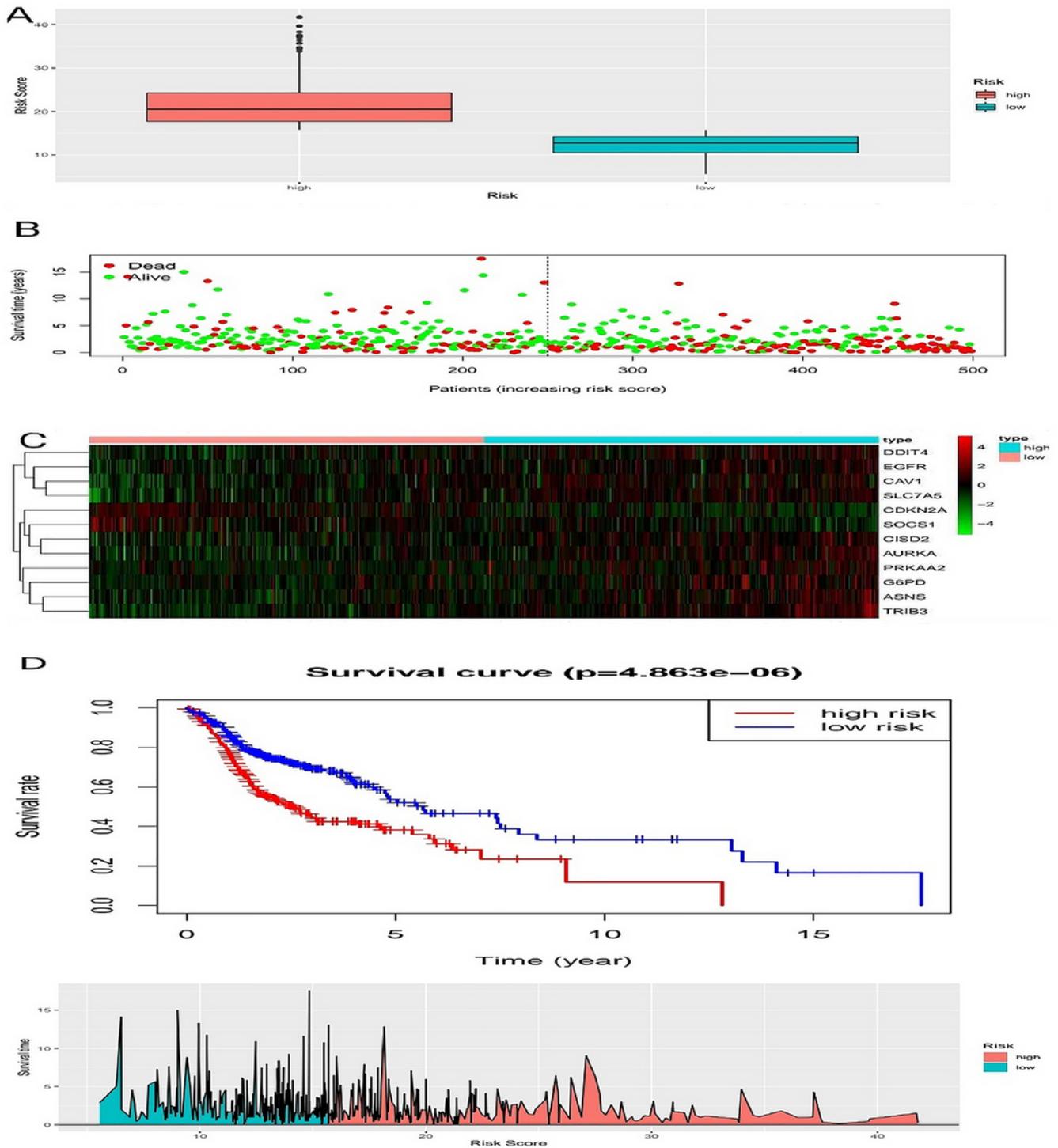


Figure 7

Construction of 12-gene signature for HNSCC prognosis. (A) The distribution of risk scores in high-risk group and low-risk group was shown. (B) We integrated the risk scores with survival analysis. Red dots indicated dead patients and green dots indicated patients alive. (C) The expression of 12 ferroptosis-related genes in both high-risk and low-risk groups was shown in heat map. (D) It was shown that the OS in high-risk group was lower than that in low-risk group by the Kaplan–Meier analysis.

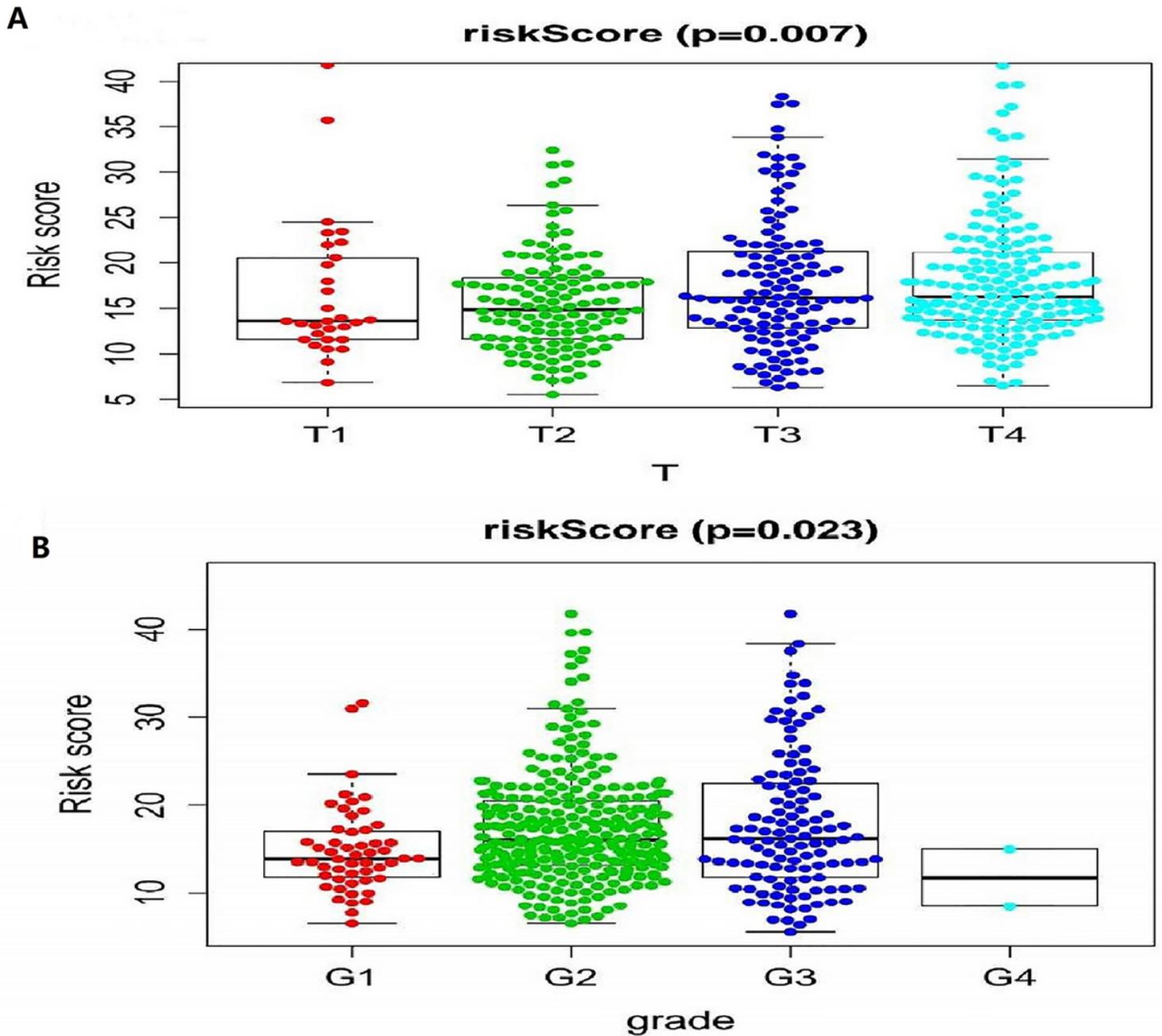


Figure 8

Association between the risk scores and clinical parameters. (A/B) Increasing risk scores were associated with higher T stages (P=0.007) and progressive tumor grades (P=0.023).

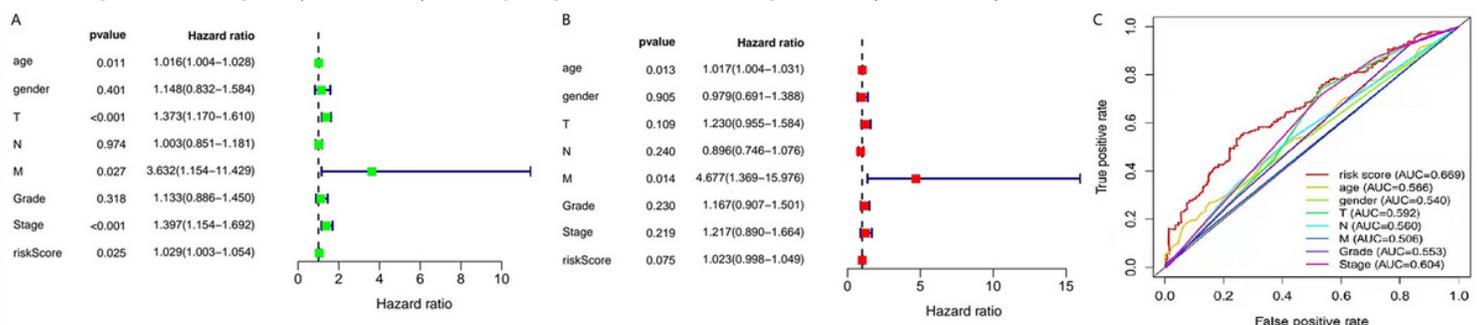


Figure 9

Evaluation of the prediction performance based on the risk scores and clinical features. (A) The relationships among OS and risk scores and clinical features, which involved in age, gender, grade, stage and TNM, were shown in the forest map by the univariate Cox analysis. (B) It was proved that the 12-gene signature was an independent prognostic indicator for HNSCC patients by the multivariate Cox analysis. (C) AUC of the model was 0.669 shown in the ROC curve.

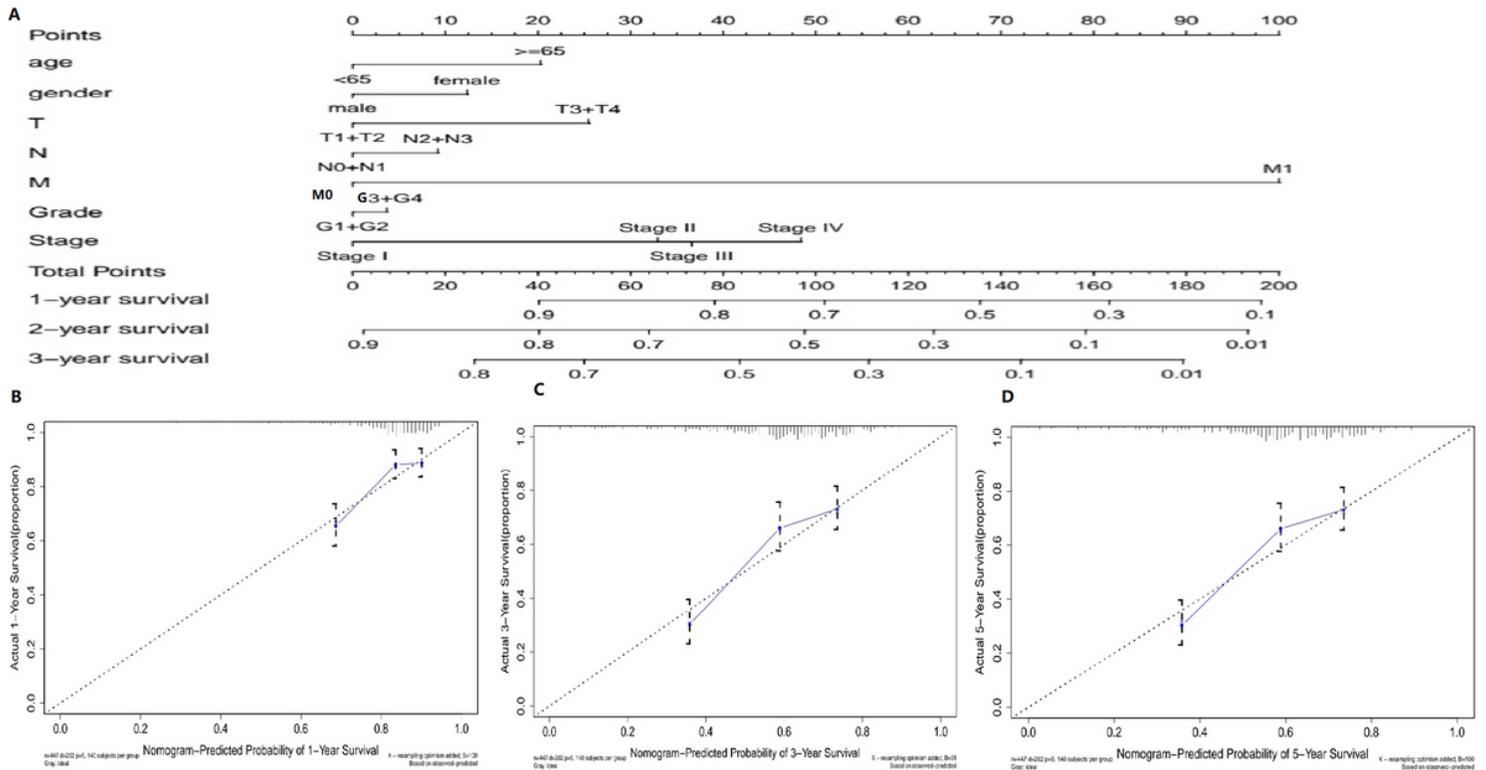


Figure 10

Construction and verification of the nomogram. (A) The nomogram for prognosis was built with risk scores and clinical features including age, gender and TNM. (B-C-D) The calibration curve for the 1-year, 3-year, 5-year OS of the nomogram.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [LiTablesSupplementalDataforFig7.exel.xlsx](#)