Original Article

Deciphering the prognostic potential of a necroptosis-related gene signature in head and neck squamous cell carcinoma: a bioinformatic analysis

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Background: Necroptosis, an alternative mode of programmed cell death (PCD) that overcomes apoptosis resistance, has been implicated in the progression and drug resistance of cancer. The aim of this study is to find the biological and prognostic significance of necroptosis in patients with head and neck squamous cell carcinoma (HNSCC).

Methods: Integrated clinical datasets from The Cancer Genome Atlas (TCGA) HNSCC cohort underwent analysis. R package "DESeq2" was used to conduct differential gene expression analysis between normal and tumor tissues in the cohort, resulting in the identification of 2,172 differentially expressed genes (DEGs). A total of 159 necroptosis-related genes (NRGs) were extracted and performed a Venn analysis to identify the optimal necroptosis-related DEGs, resulting in the selection of 25 genes specifically associated with necroptosis in HNSCC. Then prognostic analyze, Cox regression analysis and prognostic model were demonstrated the ability to predict the extent of immunological infiltration in HNSCC.

Results: Among these DEGs, five genes (*FADD*, *H2AZ1*, *PYGL*, *JAK3*, and *ZBP1*) were found to have prognostic value (P<0.05). Then, bioinformatic analyses were conducted, and the biological and clinical significance of these five genes were demonstrated. Furthermore, Cox regression analysis was performed to develop a prognostic gene model based on these genes, which effectively classified HNSCC patients into low- or high-risk groups. The prognostic model also demonstrated the ability to predict the extent of immunological infiltration in HNSCC. Additionally, a predictive nomogram based on the clinicopathological features of these five prognostic DEGs was constructed.

Conclusions: We performed a systematic bioinformatic analysis to identify necroptosis-related prognostic genes in HNSCC patients. These genes' prognostic value was synthesized into a predictive nomogram for forecasting HNSCC progression.

Keywords: Head and neck squamous cell carcinoma (HNSCC); apoptosis resistance; necroptosis; bioinformatics

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the most prevalent malignant lesion in the head and neck region, as reported by the latest global incidence data (1). Despite advances in therapy, the prognosis of HNSCC patients, particularly those diagnosed at advanced stages, continues to be impacted by recurrence and metastasis (2,3). Similar to cancers in other anatomical locations, HNSCC displays heterogeneous and complex biological characteristics (4). Among these changes, resistance to apoptosis has been identified as a significant cellular obstacle that limits the efficacy of radiotherapy and chemotherapy against HNSCC (5,6). The underlying biological mechanisms responsible for apoptosis resistance have been extensively investigated in recent literature. However, overcoming this resistance remains an essential and active area of research in HNSCC treatment.

It is well-recognized that apoptosis is a normal programmed cell death (PCD) mechanism. Cancer cells possess the ability to induce or inhibit PCD, thereby acquiring non-apoptotic forms (7). In recent years, a novel form of PCD known as necroptosis has been unveiled, which has been shown to impede cancer progression (8-10). Necroptosis can be triggered by the activation of surface-associated death receptors and is typically mediated by receptor-interacting serine/threonine kinase (RIPK)1, RIPK3, and mixed lineage kinase domain-like pseudo kinase (MLKL) (11). During necroptosis, necroptotic cells release various intracellular contents and biomolecules into the extracellular fluid, thereby impacting the microenvironment surrounding cancer cells (9). Although necroptosis may facilitate tumor growth and T cell death, it is also associated with anti-tumor immune responses. Recent studies have explored the correlation between necroptosis-related genes (NRGs) and prognosis, as well as immune infiltration in diverse cancers such as soft tissue sarcoma and breast cancer (10-12). It has been demonstrated that necroptosis-related scores can predict improved prognosis and heightened immune infiltration in these cancers. Necroptosis also represents a crucial cellular response capable of regulating cancer onset, progression, and dissemination. Researchers suggest that prognostic markers for specific cancers and diseases may include necroptosis regulatory factors (9). Additionally, NRG integration into models has been utilized to forecast overall survival (OS) in HNSCC patients. Despite serving as prognostic indicators for certain cancers and diseases, the molecular mechanisms through which necroptosis regulatory factors impact HNSCC outcomes remain incompletely understood (13).

Nevertheless, despite being considered an alternative form of PCD that overcomes apoptosis resistance, the clinical relevance of necroptosis in cancer remains inconclusive (7,14). Moreover, different NRGs have been found to display contradictory roles in different types of cancers, making it difficult to establish their functions in HNSCC. Considering this, the objective of our study was to elucidate the clinical and prognostic significance of cellular necroptosis in HNSCC. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-743/rc).

Highlight box

Key findings

- The biological and clinical significance of five necroptosis-related genes (*FADD*, *H2AZ1*, *PYGL*, *JAK3*, and *ZBP1*) were demonstrated. The ability of those genes was identified to predict the extent of immunological infiltration in head and neck squamous cell carcinoma (HNSCC).

What is known and what is new?

- Necroptosis, an alternative mode of programmed cell death that overcomes apoptosis resistance, has been implicated in the progression and drug resistance of cancer.
- The biological and prognostic significance of necroptosis in patients with HNSCC was demonstrated.

What is the implication, and what should change now?

- These genes' prognostic value was synthesized into a predictive nomogram for forecasting HNSCC progression.

Methods

Datasets and preprocessing

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). In this study, we utilized the RNA-sequencing (RNA-seq) expression profiles and corresponding clinical information for HNSCC obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) as of January 20, 2022. A total of 504 HNSCC patients were examined in this study, those lacking information regarding OS were excluded. Using the R package "DESeq2", we conducted differential gene expression analysis between normal and tumor tissues in the TCGA HNSCC cohort, resulting in

the identification of 2,172 differentially expressed genes (DEGs). Based on prior literature reviews (7,15), we extracted a total of 159 NRGs. Subsequently, we performed a Venn analysis to identify the optimal necroptosis-related DEGs, resulting in the selection of 25 genes specifically associated with necroptosis in HNSCC (table available at https://cdn.amegroups.cn/static/public/tcr-24-743-1.pdf).

Analysis for the genetic alterations of the filtered NRGs in HNSCCs

To assess the expression levels of the selected necroptosis-related DEGs between normal tissues and HNSCC, we utilized the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia2.cancer-pku.cn). Additionally, we obtained information on the genetic alterations of the NRGs in patients with HNSCC from the cBioPortal for Cancer Genomics (http://www.cbioportal.org), specifically using data from the TCGA cohort (16). We further analyzed the impact of these genetic alterations on OS and disease-specific survival (DSS).

Functional enrichment analysis

To assess the functional roles of the filtered NRGs, Gene Ontology (GO) analysis was conducted, encompassing biological process, cellular component, and molecular function categories. This analysis was carried out using the "ggplot2" package in the R software. Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed to examine the biological functions and pathways associated with the filtered genes.

Prognostic analysis

For HNSCC patients, the prognostic significance of the filtered genes was evaluated using the Kaplan-Meier method. OS was assessed based on the expression levels of each of the filtered genes. The analysis included calculation of hazard ratios (HRs) along with 95% confidence intervals (CIs), as well as log-rank P values, which were presented to determine the significance of the results.

Prediction for the cancer stem-like cell (CSC) potential and drug sensitivity

To assess the stemness potential of HNSCC, messenger RNA (mRNA) intrinsic subtype (mRNAis) scores were calculated using the one-class logistic regression (OCLR) machine-learning algorithm (17). Subsequently, Spearman correlations were analyzed to evaluate the correlation between the expression levels of the filtered genes and the CSC potential in HNSCC. To further investigate the relationship between the expression levels of the filtered genes and drug sensitivities, we utilized the Genomics of Drug Sensitivity in Cancer (GDSC) database (https://www.cancerrxgene.org/) (18). This allowed us to evaluate the potential correlations between the expression levels of the filtered genes and the sensitivities of HNSCCs to various drugs.

Immune infiltration analysis and immune checkpoint/immunotherapy response prediction

To investigate the correlations between the filtered NRGs and immune infiltration, we utilized the Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/). This resource aided in analyzing and summarizing the associations between the NRGs and immune infiltration. The results were presented using the "pheatmap" package in the R software. Next, we predicted immune checkpoint status by assessing the correlations between the filtered genes and eight immune checkpoint-related genes namely, *SIGLEC15*, *TIGIT*, *CD274*, *HAVCR2*, *PDCD1*, *CTLA4*, *LAG3*, and *DCD1LG2*. Subsequently, we employed the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to predict the response to cancer immunotherapy. The results of this analysis were visualized using the "ggplot2" and "ggpubr" packages in the R software (19).

Development of the NRGs prognostic model

We randomly allocated HNSCC patients into training and testing sets at a ratio of 7:3 to develop the prognostic NRG model. To mitigate overfitting and enhance model robustness, we employed the least absolute shrinkage and selection operator (LASSO) Cox analysis method. LASSO is particularly effective in selecting subsets of potential predictive variables, especially when the variable set is large. The TCGA HNSCC patients were subsequently divided into low- and high-risk subgroups based on the median risk score derived from the model. Kaplan-Meier analysis was performed to compare the OS rates between these two subgroups. For Kaplan-Meier curves, P values and HRs with 95% CIs were generated by log-rank tests

and univariate Cox proportional hazard regression. The predictive accuracy of each filtered gene and the risk score was evaluated through time receiver operating characteristic (ROC) analysis. Immune infiltration analysis was performed to compare the immune infiltration status between the low- and high-risk subgroups. To further analyze the association between the clinical characteristics and the risk score, univariate logistic and multivariable Cox regression models were employed. A forest was used to show the P value, HR, and 95% CI of each variable through the "forestplot" R package. Additionally, a predicted nomogram was constructed to predict the 1-, 3-, and 5-year OS rates.

Statistical analysis

In this study, all analysis methods and R packages were implemented using the R Foundation for Statistical Computing [2020], specifically version 4.0.3. For statistical significance, a P value less than 0.05 was considered statistically significant.

Results

Defining of NRGs in HNSCCs

A Venn analysis identified a total of 25 genes that were filtered between all the DEGs in HNSCCs and the reviewed NRGs (*Figure 1A*). The expression levels of these 25 filtered NRGs were then compared between HNSCCs and normal mucosal tissues using the TCGA HNSCC dataset (*Figure 1B*). It was observed that all the filtered genes showed a significantly increased expression pattern in HNSCCs compared to normal mucosal tissues (P<0.001). Subsequently, the genetic alterations in the filtered NRGs were investigated in HNSCCs using the cBioPortal software. The frequency of gene alterations, including mutations, structural variants, amplifications, deep deletions, mRNA highs, and mRNA lows were demonstrated in *Figure 1C,1D*. It was found that the genetic variants for the filtered NRGs occurred at a low rate, while more alterations were observed in the mRNA expressions in HNSCCs. The prognostic roles of NRG alterations in patients with HNSCCs were also analyzed. Significant correlations were observed between the presence of alterations and OS or DSS (P=0.045 and P=0.03, respectively, *Figure 1E,1F*). These data suggest that the prognostic values of the NRGs might mostly be attributed to their expression levels, i.e., protein levels, in HNSCCs.

Functional enrichment analyses of the filtered NRGs in HNSCCs

To gain insights into the functions of the filtered NRGs in HNSCCs, pathway analysis was performed using the GO and KEGG databases. The analysis revealed that the translated proteins of the 25 filtered genes were primarily localized in the cellular cytosol (*Figure 2A*). These proteins were found to be actively involved in the apoptotic process and exerted their influences through binding with other cytosol proteins (*Figure 2B,2C*). Furthermore, the KEGG pathway analysis indicated that the 25 filtered NRGs were most significantly associated with the apoptosis pathway (*Figure 2D*). This suggests that these genes play a crucial role in regulating apoptosis processes in HNSCCs.

Prognostic analysis for the filtered NRGs in HNSCCs

Cox regression analysis was conducted to assess the prognostic values of the 25 filtered necroptosis-related DEGs (*Figure 3A*). From this analysis, a total of five genes were identified to have statistically significant prognostic values. Specifically, it was observed that high expressions of *FADD* (*Figure 3B*), *H2AZ1* (*Figure 3C*), and *PYGL* (*Figure 3D*) were associated with significantly decreased OS rates in HNSCC patients. On the other hand, low expressions of *JAK3* (*Figure 3E*) and *ZBP1* (*Figure 3F*) were also associated with significantly decreased OS rates in HNSCC patients. These findings suggest that the expression levels of these five genes have prognostic implications for HNSCC patients.

The biological and clinical significance of the five filtered NRGs in HNSCCs

To investigate the potential correlations between the five filtered NRGs and CSC potential, an analysis was performed. It was found that alterations in the expressions of the five filtered NRGs were significantly correlated with the CSC potential of HNSCCs compared to normal mucosal tissues (*Figure 4A*). Additionally, predictions of targeted drug sensitivity were demonstrated based on the different expression trends of these NRGs in Cancer Therapeutics Response Portal (CTRP) database (*Figure 4B*).

The immunological correlations of the 5 filtered genes were also explored, considering the important role of the immunological microenvironment in the progression of HNSCCs. The most significant correlations were observed for *ZBP1*, *PYGL*, and *JAK3*. High expressions of

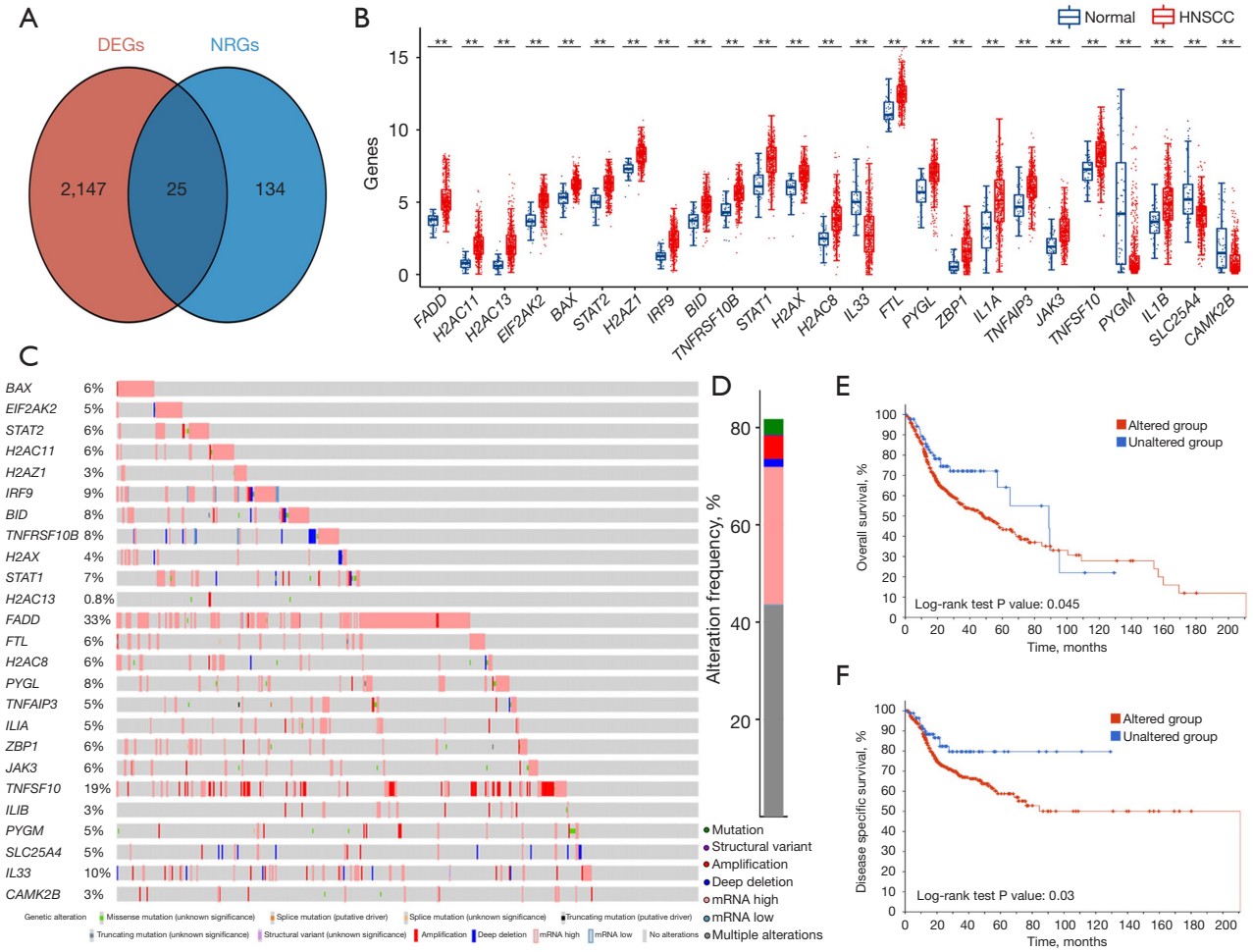


Figure 1 Landscape of expression and genetic variation of differentially expressed NRGs in HNSCC. (A) Venn plot of the identified necroptosis-related DEGs. (B) Significant expression of filtered genes in HNSCC and the compared normal tissues. (C) The mutation frequency and classification of 25 filtered genes in the HNSCC cohort. (D) A summary for all the genetic alteration of filtered genes in HNSCC. (E) OS analysis based on the genetic alterations of the filtered NRGs in HNSCC patients. (F) DSS analysis based on the genetic alterations of the filtered NRGs in HNSCC patients. **, P<0.01. DEGs, differentially expressed genes; NRGs, necroptosis-related genes; HNSCC, head and neck squamous cell carcinoma; mRNA, messenger RNA; OS, overall survival; DSS, disease-specific survival.

ZBP1 and *JAK3* were positively correlated with increased infiltrations of immunological cells in HNSCCs, while a negative correlation was observed for *PYGL* expression and immunological cell infiltration (*Figure 5A*). Moreover, immune checkpoint analysis revealed that low expressions of *FADD* significantly influenced the expressions of *PDCD1* and *TIGIT*, while low expressions of *PYGL* correlated with expressions of *CTLA4*, *HAVCR2*, *LAG3*, *PDCD1*, *TIGIT*, and *SIGLECI5* (*Figure 5B,5C*). High expressions of *JAK3* and *ZBP1* were also closely associated with expressions of immune checkpoint-related molecules (*Figure 5D,5E*). Furthermore, cancer immunotherapy responses could possibly be forecasted through the analysis of these NRGs. Increased TIDE scores were observed in HNSCC cases with high expressions of *FADD* and *JAK3*, as well as in cases with low expressions of *ZBP1* (*Figure 5F-5K*). These findings suggest that the expressions of these 5 NRGs are correlated with CSC potential, immune infiltration status, and potential response to immunotherapy in HNSCCs.

Construction of an NRG-related prognostic gene model

Cox regression analysis was performed to develop a prognostic gene model using the five filtered prognostic

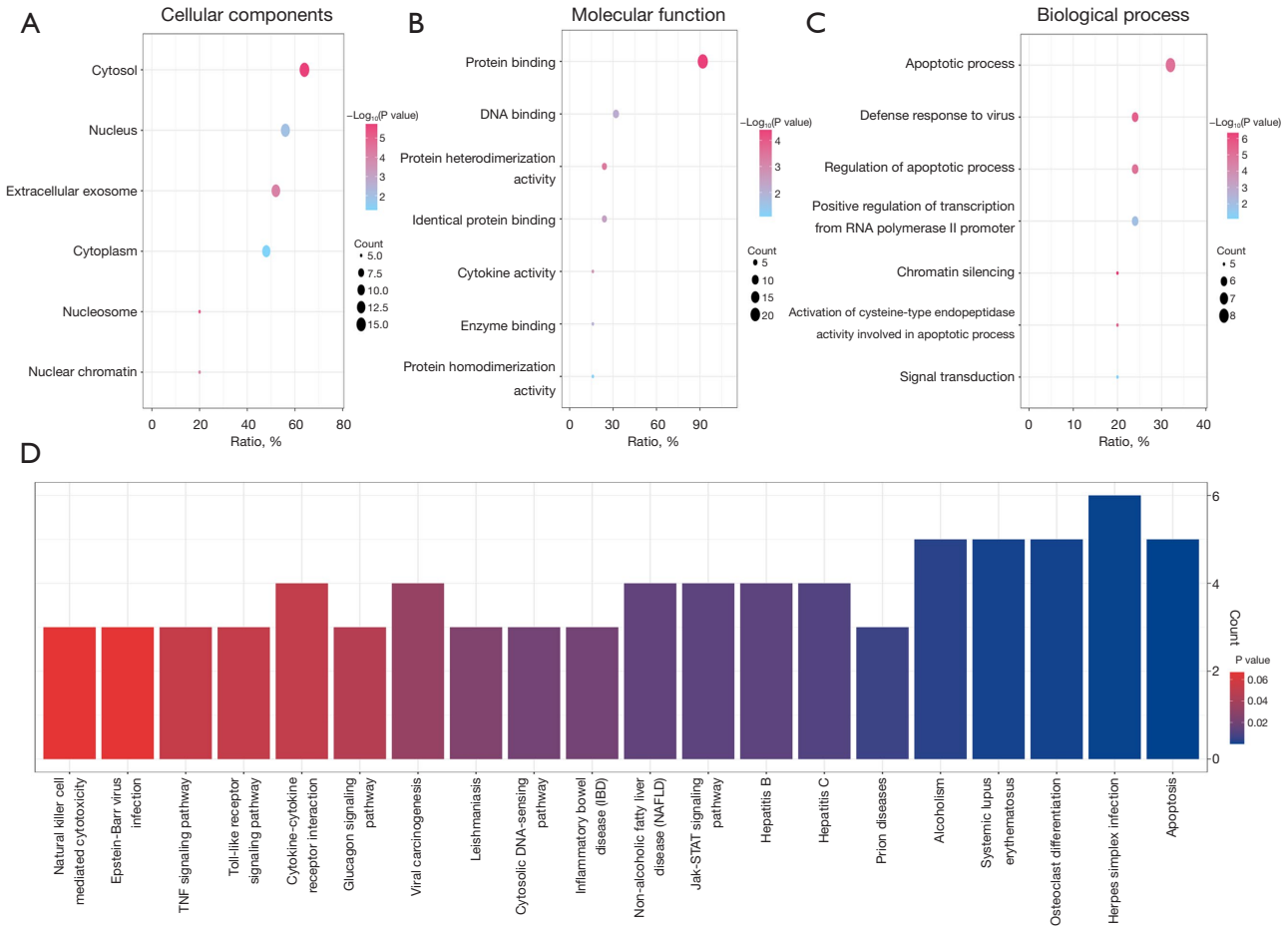


Figure 2 Enrichment analysis of the filtered NRGs in HNSCC. GO enrichment analysis predicted the functional roles of targeted genes based on three aspects, including (A) cellular components; (B) molecular function; (C) biological processes; (D) KEGG analysis for the filtered genes. NRGs, necroptosis-related genes; HNSCC, head and neck squamous cell carcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

NRGs (*Figure 6A,6B*). The risk score was calculated using the following formula: risk score = (0.2359) × *H2AZ1* + (0.1204) × *FADD* + (0.1572) × *PYGL* + (–0.0987) × *ZBP1* + (–0.0759) × *JAK3*. Based on the risk scores, HNSCC patients were divided into low- and high-risk subgroups. The distribution of risk scores, survival status, and expression levels of the five NRGs were presented (*Figure 6C*). The OS rates demonstrated an inverse correlation with the increase in NRG-related risk scores (*Figure 6C*). The Kaplan-Meier curve showed that high-risk scores were associated with significantly poorer OS rates in HNSCC patients (median time: 2.7 *vs.* 5.2 years, P<0.001; *Figure 6D*). The NRG-based prognostic model exhibited predictive area under the curve (AUC) values of 0.630, 0.658, and 0.624 in the 1-, 3-, and 5-year ROC curves, respectively (*Figure 6E*). Furthermore, the correlations between the prognostic model and immunological cellular infiltrations were investigated (*Figure 7A-7F*). The NRG-based prognostic model was found to be capable of predicting the status of cellular immunological infiltrations in the microenvironment surrounding HNSCC cells. These results indicate that the NRG-related prognostic model can effectively predict the survival outcomes and immunological status of HNSCC patients.

Building a predictive nomogram based on NRG-related risk stratifications

The clinicopathological features were examined for their correlations with the five filtered NRGs. Univariate

A

NRGs	P value	HR	Low 95% CI	High 95% CI
BAX	0.66353441	0.94246597	0.72164855	1.23085136
EIF2AK2	0.73364847	1.04741343	0.80211988	1.36771938
STAT2	0.51729904	0.91541579	0.70055299	1.196178
H2AC11	0.08173042	1.26956137	0.97038854	1.66096982
H2AZ1	0.02496899	1.36499602	1.03990308	1.79171903
IRF9	0.07031667	0.78133621	0.59812035	1.02067466
BID	0.38234713	1.12659907	0.86222293	1.47203864
TNFRSF10B	0.44982655	1.10933333	0.84759994	1.45188831
H2AX	0.71025177	0.95069936	0.72812993	1.2413022
STAT1	0.19244684	0.83646696	0.63948493	1.09412581
H2AC13	0.92624839	1.01266469	0.77577771	1.32188609
FADD	0.00032105	1.6464963	1.2548215	2.16042686
FTL	0.08123404	1.27030386	0.97074258	1.6623067
H2AC8	0.67722491	0.9448738	0.72346776	1.2340377
PYGL	0.02451939	1.3616452	1.04045655	1.78198471
TNFAIP3	0.419226	1.11634113	0.85471683	1.45804725
ILIA	0.13127707	1.23013949	0.93999114	1.60984832
ZBP1	0.00108532	0.6361268	0.48495262	0.83442648
JAK3	0.01067355	0.70407496	0.53782867	0.92170905
TNFSF10	0.90776845	1.01595382	0.77729015	1.32789816
ILIB	0.18538182	1.19795161	0.91696942	1.56503372
PYGM	0.96488961	0.99401644	0.76092154	1.29851585
SLC25A4	0.05512476	1.30058995	0.9942512	1.70131474
IL33	0.70282827	0.94935911	0.7269106	1.2398811
CAMK2B	0.16816449	1.2066103	0.92376771	1.57605467

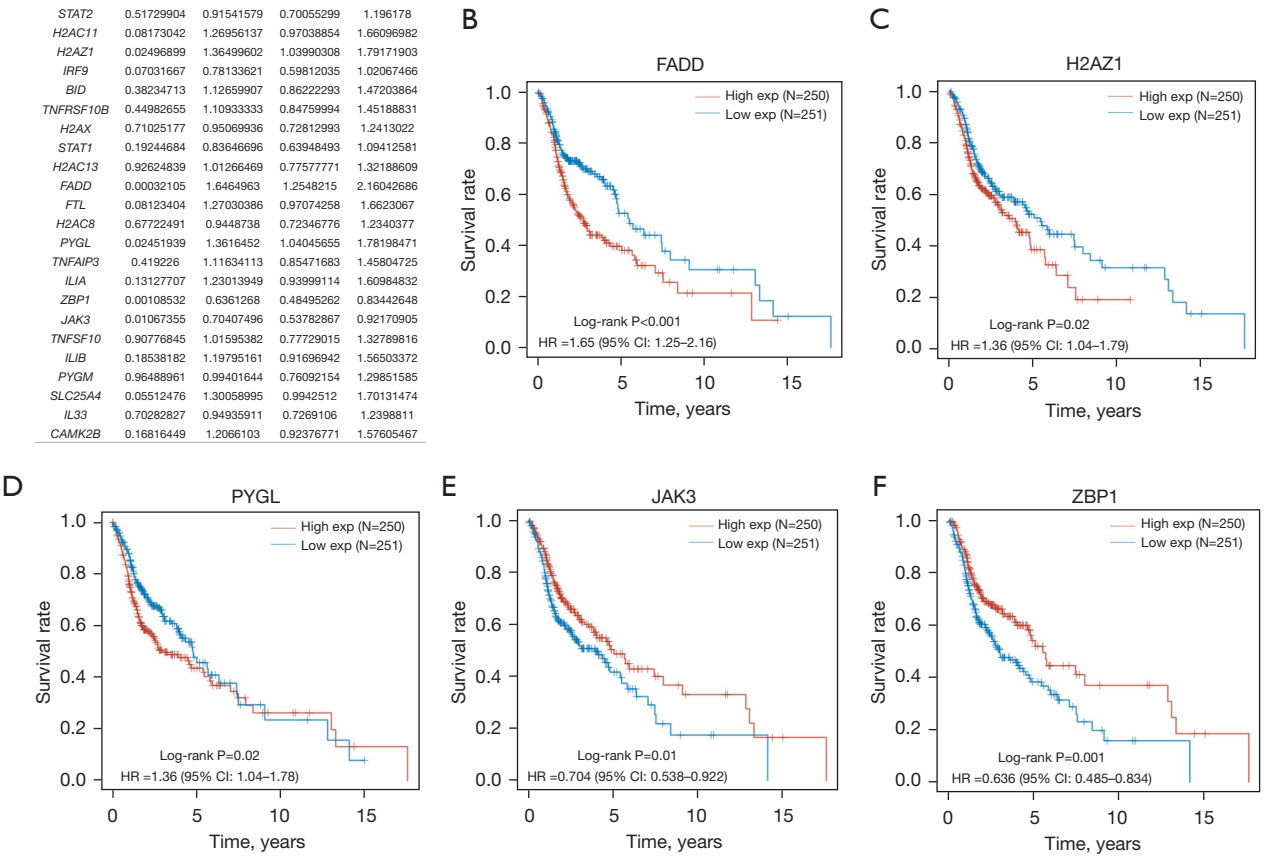


Figure 3 The prognostic value of the filtered NRGs in HNSCC. (A) A summary for the OS analysis for all the filtered NRGs in HNSCC. (B) The OS curve of *FADD*. (C) The OS curve of *H2AZ1*. (D) The OS curve of *PYGL*. (E) The OS curve of *JAK3*. (F) The OS curve of *ZBP1*. NRGs, necroptosis-related genes; HR, hazard ratio; CI, confidence interval; HNSCC, head and neck squamous cell carcinoma; OS, overall survival.

and multivariate analyses were performed to identify independent factors affecting the prognosis of HNSCC patients. The expressions of *H2AZ1*, *FADD*, and *PYGL*, as well as age, pN stage, and pM stage, were found to be independent factors influencing the prognosis of HNSCC patients (*Figure 8A,8B*). Based on these findings, a predictive nomogram was constructed to accurately predict the 1-, 3-, and 5-year OS rates for HNSCC patients (*Figure 8C,8D*). The nomogram integrates the identified independent factors, providing a visual tool for predicting the survival outcomes of patients.

Discussion

Nowadays, the primary treatment options for HNSCC are surgery, chemoradiotherapy, and targeted or immunotherapies (20). However, in cases of advanced HNSCC, the development of cancer cell resistance to apoptosis, leading to cancer cell repopulation, is a major factor contributing to therapeutic failure (21-23). To address this, there is a need to induce cancer cell death by exploring novel apoptotic pathways (24). Necroptosis has recently emerged as a promising target to overcome apoptosis resistance in cancer cells (14,25). Initially discovered due to its involvement in inflammatory cell death (26,27), necroptosis plays a complex role in initiating PCD, overcoming apoptosis resistance, and activating necroptosis-related immune responses in cancer (7,24,28,29). Tumor necroptosis has also been widely observed in HNSCC (15). In this study, a series of bioinformatics analyses were

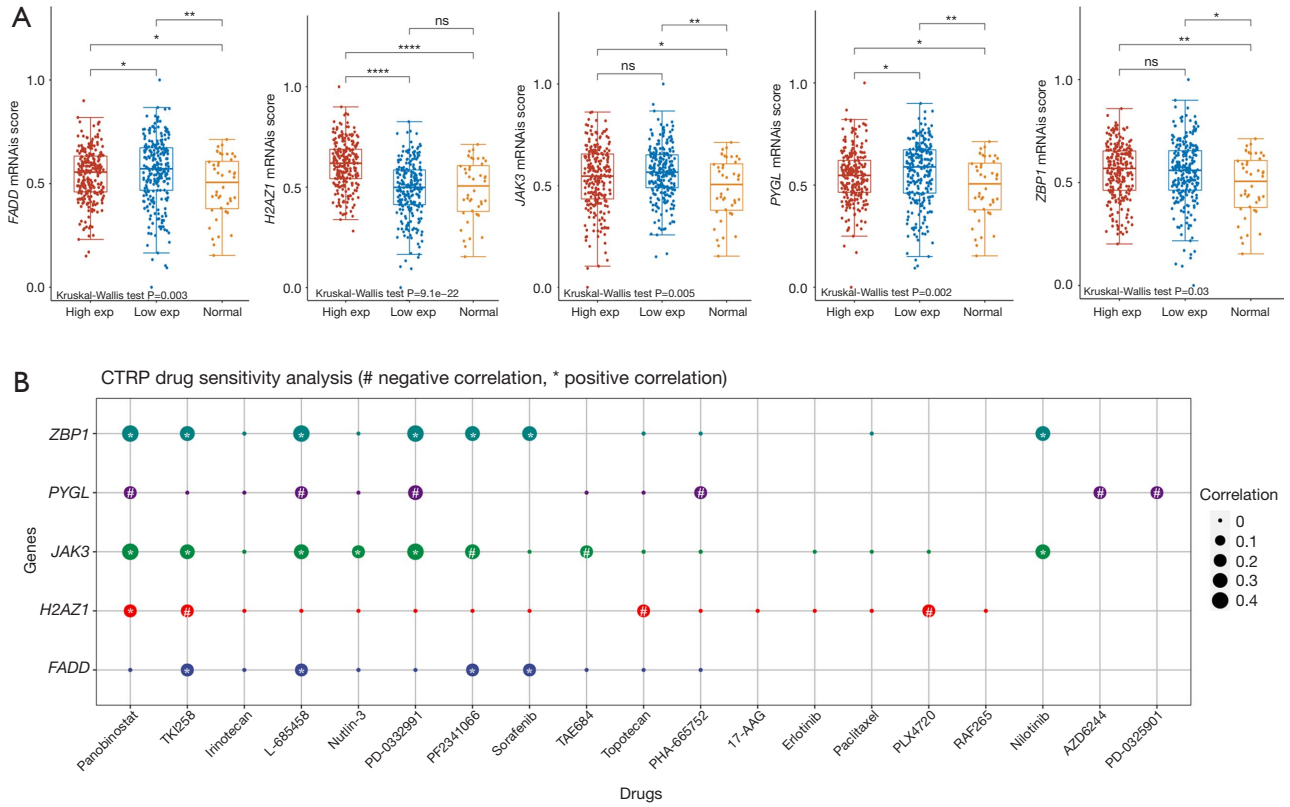
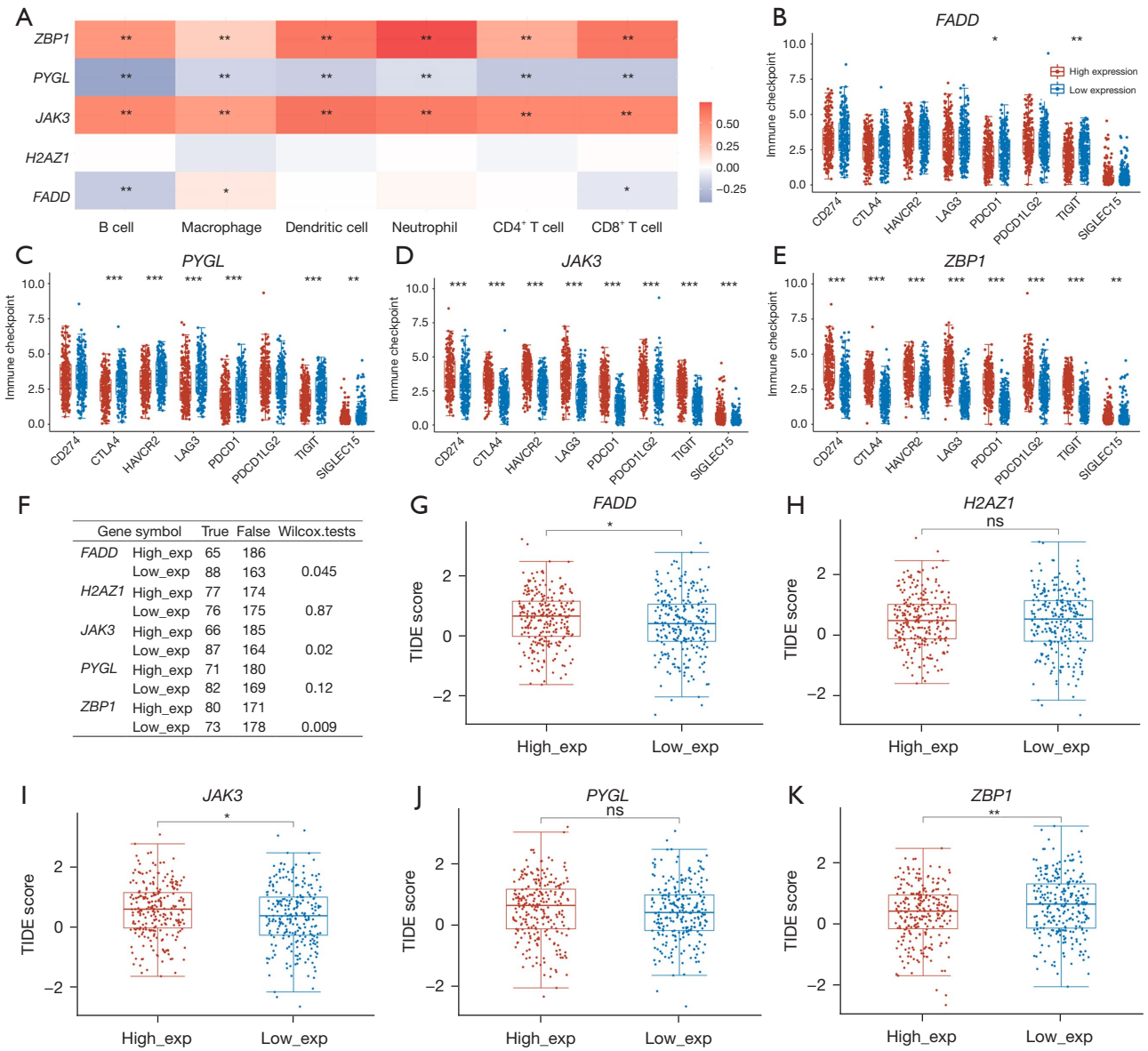


Figure 4 Further analysis for the CSCs potential and drug sensitivity based on the expression alterations of the five filtered NRGs-related DEGs. (A) Correlations of the five filtered NRGs-related DEGs to the CSCs potential in HNSCC. (B) Prediction for drug sensitivity based on the five filtered NRGs-related DEGs in CTRP database. *, P<0.05; **, P<0.01; ****, P<0.001; ns, P>0.05. mRNAis, mRNA intrinsic subtype; mRNA, messenger RNA; exp, expression; CSCs, cancer stem-like cells; NRGs, necroptosis-related genes; DEGs, differentially expressed genes; HNSCC, head and neck squamous cell carcinoma; CTRP, Cancer Therapeutics Response Portal; ns, not significant.

performed to investigate the prognostic significance of cellular necroptosis in HNSCC patients. By examining the functions and correlations of NRGs, the study aimed to gain insights into the potential implications of cellular necroptosis in predicting prognosis and guiding treatment strategies for HNSCC patients.

In this study, we investigated the expression levels and prognostic values of NRGs in HNSCC. We found that high expressions of *FADD*, *H2AZ1*, and *PYGL*, as well as low expressions of *JAK3* and *ZBP1*, were associated with poor survival in HNSCC patients. Although several prognostic models have been developed to predict OS or DSS in HNSCC, we proposed a novel prognostic gene model based on the five filtered prognostic NRGs. We believe that considering the potential functions of NRG-translated proteins is an important addition to existing prognostic models for HNSCC patients, particularly in advanced stages, as necroptosis plays critical roles in cellular apoptosis in HNSCC (30-32). By incorporating the molecular information related to necroptosis, our prognostic gene model may provide a more comprehensive understanding of the prognosis and guide the treatment strategies for HNSCC patients.

The five filtered prognostic NRGs identified in this study have important functions in the process of cellular necroptosis. The overexpression of some NRGs has initially been reported to serve as adaptor molecules for receptor-mediated apoptosis in various types of cancers (33). Among these five NRGs, the role of *FADD* in necroptosis has been mentioned, particularly in the context of chronic inflammatory signals during the proliferation of liver cancer cells (34). *H2AZ1* has been associated with tumor malignancy by regulating the cellular cycle in hepatocellular carcinoma and lung adenocarcinoma (35-37). *JAK3* plays a crucial



Gene symbol		True	False	Wilcox.tests
FADD	High_exp	65	186	
	Low_exp	88	163	0.045
H2AZ1	High_exp	77	174	
	Low_exp	76	175	0.87
JAK3	High_exp	66	185	
	Low_exp	87	164	0.02
PYGL	High_exp	71	180	
	Low_exp	82	169	0.12
ZBP1	High_exp	80	171	
	Low_exp	73	178	0.009

Figure 5 The association between five-filtered prognostic NRGs and TIMER, and prediction for the cancer immunotherapy responses based on the five-filtered prognostic NRGs. (A) A summary for the association between the filtered prognostic NRGs and immune infiltration. (B-E) The association between each filtered prognostic NRGs (*FADD*, *PYGL*, *JAK3*, or *ZBP1*) and immune checkpoints, respectively. (F-K) Prediction for the cancer immunotherapy responses based on the five-filtered prognostic NRGs. *, P<0.05; **, P<0.01; ***, P<0.005; ns, P>0.05. TIDE, Tumor Immune Dysfunction and Exclusion; exp, expression; NRGs, necroptosis-related genes; TIMER, Tumor Immune Estimation Resource; ns, not significant.

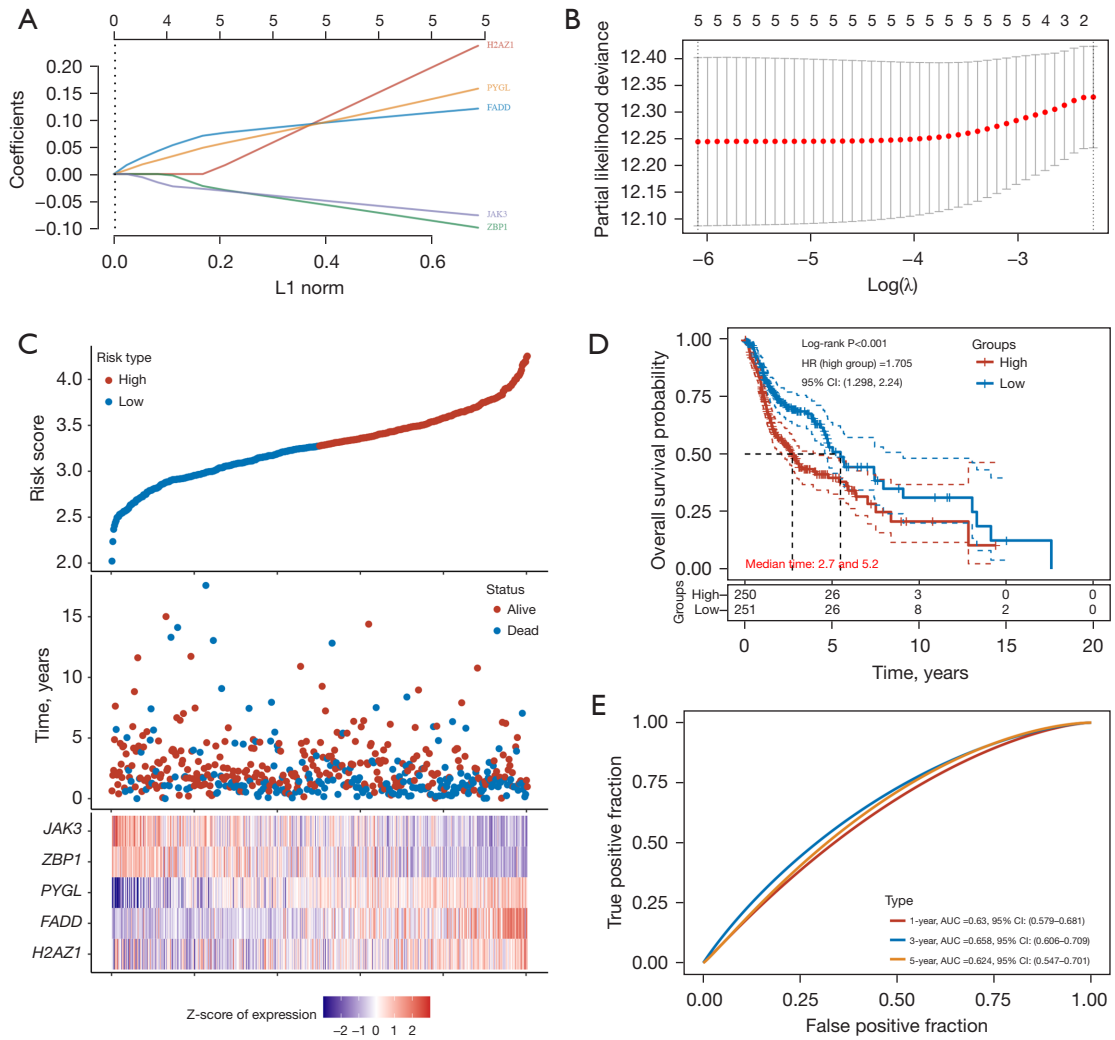


Figure 6 Construction of a prognostic NRGs model. (A) LASSO coefficient profiles of the five-filtered prognostic NRGs. (B) Plots of the ten-fold cross-validation error rates. (C) Distribution of risk score, survival status, and the expression of the five-filtered prognostic NRGs. (D,E) OS curves for HNSCC patients in the high/low-risk group and the ROC curve of measuring the predictive value. HR, hazard ratio; CI, confidence interval; AUC, area under the curve; NRGs, necroptosis-related genes; LASSO, least absolute shrinkage and selection operator; HNSCC, head and neck squamous cell carcinoma; OS, overall survival; ROC, receiver operating characteristic.

role as a regulator or receptor for cytokine signals during cancer cell progression (38,39). The up-regulation of *PYGL*, another NRG-translated protein, has been extensively investigated in a *RIP3*-dependent manner in colorectal cancer, highlighting its involvement in cellular necroptosis (40,41). *ZBP1* has been reported to mediate tumor cellular necroptosis in breast cancer, and its deletion can inhibit tumor necroptosis, promoting cancer cell growth (42). Furthermore, *ZBP1* is required for both type I (β) and type II (γ) interferon-induced necroptosis, which can potentially interfere with cancer treatment in its absence (43).

Necroptosis often occurs when caspase-mediated apoptosis is impaired, making it an attractive target for overcoming apoptosis resistance in cancer (7,44). Furthermore, necroptosis is known to induce a strong pro-inflammatory response and can elicit adaptive immune

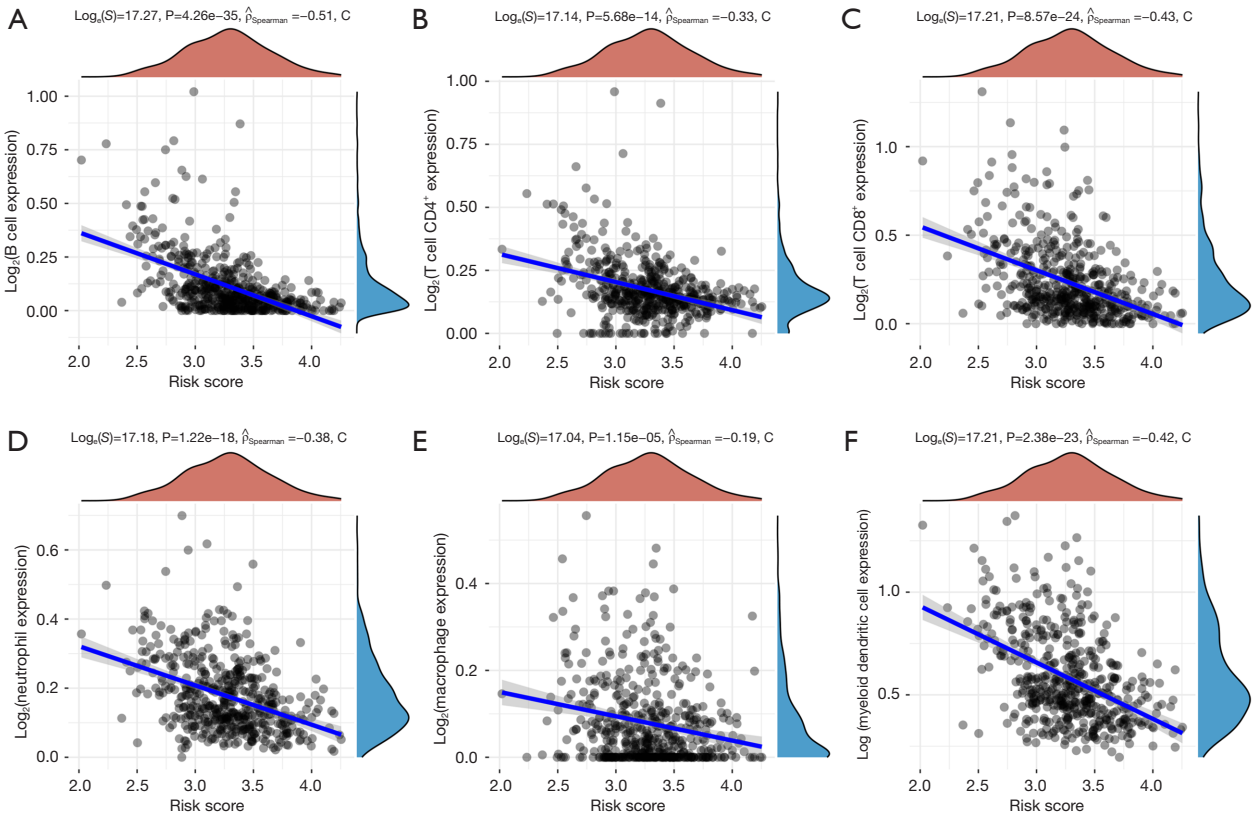


Figure 7 Further analysis for the correlations between immune infiltration status and risk scores based on the prognostic model. (A-F) For B cell, CD4$^+$ T cell, CD8$^+$ T cell, neutrophil, macrophage, and myeloid dendritic cell, respectively.

responses *in vivo* (7,28). Therefore, the concept of a necroptosis-associated immunological microenvironment has been emerging (27). In this study, we also investigated the correlations between the five filtered NRGs and immune infiltrations in HNSCC. These findings suggest the existence of a complex relationship between necroptosis and immune responses in HNSCC. This information provides valuable insights into the potential use of NRG-related targets for future immune checkpoint inhibition therapies in HNSCC. By targeting the pathways associated with necroptosis, it may be possible to modulate the immune microenvironment and enhance the efficacy of immune checkpoint inhibitors in HNSCC.

In our study, we conducted all analyses using the TCGA HNSCC cohort. However, to validate our findings, it would be advantageous to verify them in a larger number of clinical samples. Additionally, there are limitations to understanding the exact NRG-related mechanisms underlying the observed prognostic changes in HNSCC. Nevertheless, our study provides valuable information that can guide further investigations into the role of necroptosis in HNSCC. Future studies can explore the specific molecular mechanisms and pathways involved in necroptosis to gain a more comprehensive understanding of its implications in HNSCC.

Conclusions

We conducted a thorough and systematic bioinformatics analysis to identify prognostic NRGs in HNSCC patients. Furthermore, we validated the predictive value of our proposed nomogram, which holds potential for clinical application. Our study provides preliminary analysis of the clinical and prognostic significance of cellular necroptosis in HNSCCs, highlighting the need for further investigations in this area. Moving forward, it is crucial to delve deeper into the mechanisms and implications of cellular necroptosis in HNSCC, to gain a more comprehensive understanding

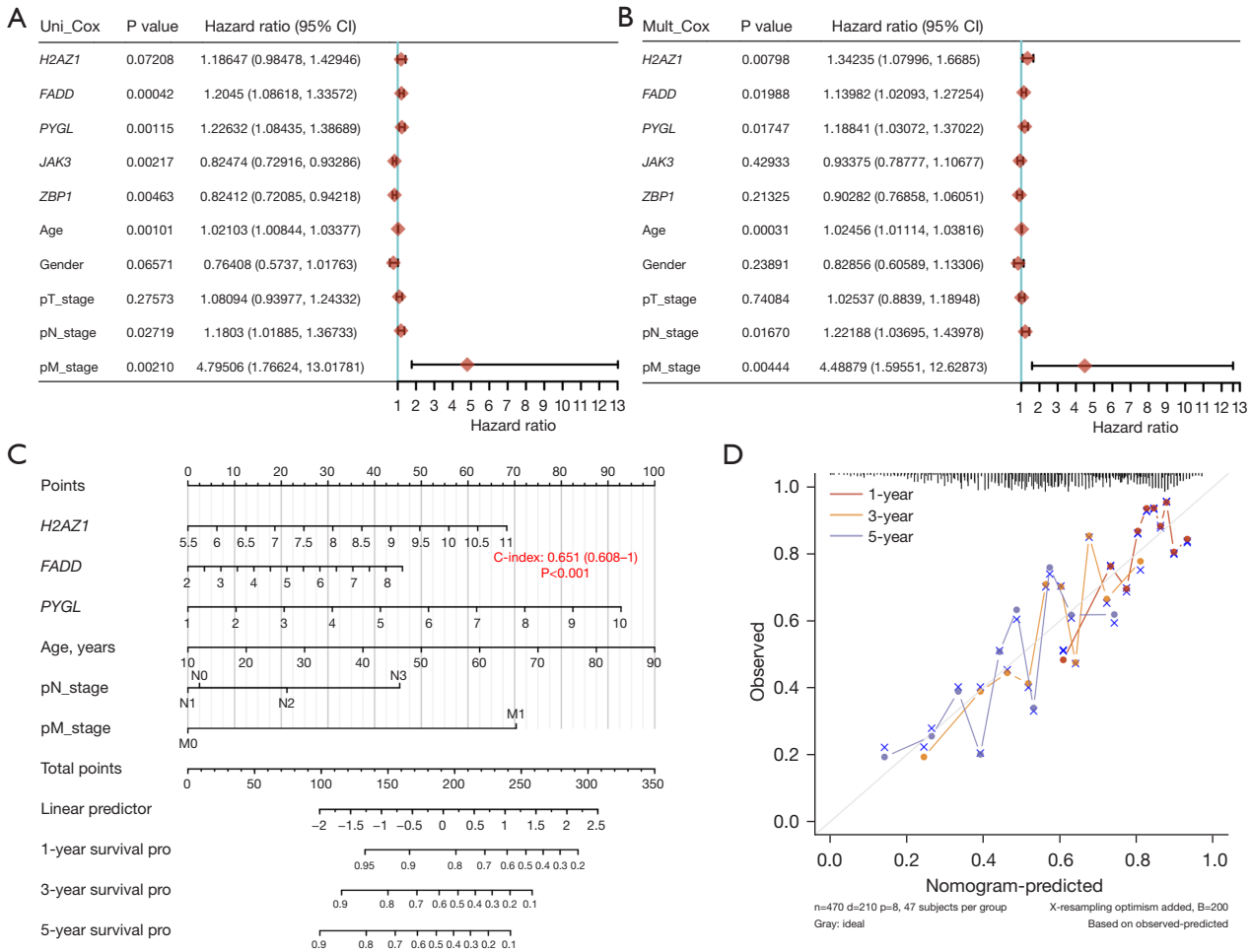


Figure 8 Construction of a predictive nomogram. (A) HR and P value of the constituents involved in univariate Cox regression considering clinical the parameters and the five-filtered prognostic NRGs in HNSCC. (B) HR and P value of the constituents involved in multivariate Cox regression considering clinical the parameters and the five-filtered prognostic NRGs in HNSCC. (C) Nomogram to predict the 1-, 3-, and 5-year OS rate of HNSCC patients. (D) Calibration curve of the OS nomogram model in the discovery group. A dashed diagonal line represents the ideal nomogram. CI, confidence interval; pro, probability; HR, hazard ratio; NRGs, necroptosis-related genes; HNSCC, head and neck squamous cell carcinoma; OS, overall survival.

of its clinical relevance.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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