Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

A novel necroptosis-related model predicts the prognosis and immunocompetence of head and neck squamous cell carcinoma

Jixia Zhang[1#], Anli Wang[1#*], Lin Qiu[2]

[1] Department of Pediatric Dentistry, Tianjin Stomatological Hospital, School of Medicine, Nankai University, Tianjin 300000, China
[2] Stomatology & National Center of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices, Peking University, Beijing 100038, China

[#] Jixia Zhang and Anli Wang contributed equally to this work.

ARTICLE INFO

Original paper

Article history:
Received: June 27, 2023
Accepted: September 23, 2023
Published: November 15, 2023

Keywords:

Head and neck squamous cell carcinoma, necroptosis, prognosis, immunity, proliferation

ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is a disease characterized by profound immunosuppression and the prognosis of HNSCC patients remains poor. Necroptosis is a programmed lytic cell-death mechanism that can promote tumor growth, angiogenesis, invasion and metastasis. The differentially expressed NRGs were screened by using the LIMMA package of R software (version 4.1.2) and the prognosis-related NRGs were obtained by COX regression analysis. We separated patients into high- and low-risk groups via the prognostic model consisting of those NRGs. The receiver operating characteristic (ROC) curve and Kaplan-Meier survival curves were used to validate the prognostic model. By bioinformatics analysis, the prognostic risk and immunocompetent models were constructed. We reevaluate the prognostic model based on the GES27020 data sets, clinicopathological variables and chemotherapeutic efficacy. Individuals in the high-risk group had much shorter overall survival (OS) times than their counterparts. Compared with clinicopathological variables, the risk model has a higher diagnostic efficiency, with the area under the ROC being 0.699. Decision Curve Analysis (DCA) showed the prognostic model-based risk score was the superior prognostic factor compared to additional indicators. Furthermore, the high- and low-risk groups had differences in immune cell infiltration and immune functions. And the CCK-8 showed that small molecular drugs could inhibit HNSCC cell proliferation in vitro. We have constructed a new necroptosis-related model, which can be used to predict the prognosis and immunocompetence of HNSCC patients and provide a theoretical basis for the study of necroptosis in the clinical prognosis of HNSCC.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.11.33

Introduction

Head and neck squamous cell carcinoma (HNSCC) comprises heterogeneous tumors that develop from the squamous epithelium of the oral cavity, the oropharynx, the larynx and the hypopharynx (1). HNSCC represents the sixth most common cancer globally with a high mortality rate of 40% to 50% (2). Regrettably, most HNSCC patients are diagnosed at an advanced stage with a poor prognosis end. Because the early symptoms of HNSCC are not obvious, between 40%-50% of the patients experiencing disease recurrence can reduce the survival to 35% even after modern, multi-modality treatments (3). Doctors are working not only to develop surgical skills, but also to research precision radiotherapy, new chemotherapy drugs and immunotherapy. However, in order to improve the prognosis of HNSCC patients, it is necessary to find more risk factors of patients. Bioinformatics technology can be used to predict the molecular markers for the prognosis of HNSCC by using gene expression from genomics. The study of potential biomarkers is of great significance for the early diagnosis, prognosis and quality of life of HNSCC patients.

Necroptosis is programmed lytic cell death that allows the release of potential immunostimulatory molecules (4). The receptor-interacting protein kinase1 (RIPK1) and 3 (RIPK3) as well as the mixed lineage kinase domain-like protein (MLKL), are necessary for the activation of the necroptosis. Necroptosis can enhance $CD8^+$ leukocyte-mediated anti-tumoral immunity by activating RIPK1 and RIPK3 within the tumor microenvironment (TME) (5). By contrast, genetic evidence supports the idea that, when activated, this cell death pathway can induce potent inflammatory responses in vivo, and thereby may induce various inflammatory diseases (6,7). In addition, many diseases are known to be correlated with necroptosis, including ischemic reperfusion injury, inflammatory, neurodegenerative, infectious, autoimmune diseases and cancer (8). Tumor necrosis is often associated with poor prognosis in patients because intratumoral necrosis in the “core” region lacks proper nutrient and oxygen access (9). Existing studies have confirmed that necroptosis can promote the metastasis of tumor cells and the death of T cells. Necroptosis could enhance pancreatic cancer cell migration and invasion by the release of C-X-C motif chemokine 5 (CXCL5) and C-X-C-motif chemokine receptor-2 (CXCR2) (10). Besides, the role of necroptosis in HNSCC is becoming more and more clear. About 50% of necrosis in HNSCC is

* Corresponding author. Email: wanganlikouqiang@icloud.com

caused by necroptosis and suggested that necroptosis can release damage-associated molecular patterns (DAMPs) to promote cancer metastasis and cancer progression (11). The necroptosis-related prognostic risk model established by Zhang Z et al. for HNSCC included 7 necroptosis-related genes (NRGs) that were significantly associated with the prognosis of HNSCC (12). However, after extracting the expression levels of 7 NRGs from the RNA-seq of HNSCC patients in the TCGA database, we found that there was no significant difference in the expression levels of VDAC1 and CHMP3 between HNSCC tissues and normal tissues, and the expression level of TRAF5 was relatively low. In addition, this study also showed that VDAC1, CHMP3 and TRAF5 do not have the high mutation rates in HNSCC. Therefore, the prognostic significance of these three genes for HNSCC patients still needs to be verified.

Above all, this study conducted a more comprehensive and systematic bioinformatics analysis of NRGs, aiming to explore the NRGs that are truly related to the prognosis of HNSCC patients. Prognostic risk and immune activity models were constructed by using prognostic-related NRGs and assessed the accuracy of the risk model. Finally, the L1000FWD database was used to obtain small-molecule targeted drugs against the necroptosis-related prognostic model, and a small molecule targeted drug was selected for in vitro experimental verification.

Materials and Methods

Data acquisition

RNA-sequencing (RNA-seq) data and clinical data of HNSCC patients were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) and GEO dataset: GSE27020. NRGs were obtained based on the KEGG website (http://www.kegg.jp/). Differentially expressed necroptosis-related genes (DE-NRGs) were screened by using the LIMMA package of R software (version 4.1.2) based on the criterion: FDR < 0.05 and |log2 (Fold Change) |> 1/2.

Functional enrichment analysis

In order to evaluate the biological functions and signaling pathways that DE-NRGs are mainly involved in, the gene mutations of all DE-NRGs were detected, and genes with a mutation rate of more than 2% were retained. Gene-level functional enrichment was performed using the Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/), and the results were visualized by the ggplot2 R package. The data sources used for this analysis included: Gene Ontology (GO): Molecular Function (MF), GO: Biological Processes (BP), GO: Cellular Components (CC), and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Establishment and validation of the prognostic model

We obtained the candidate NRGs correlated with the prognosis of HNSCC by univariate Cox regression analysis ($P < 0.05$) and the hazard ratios (HR) with 95% confidence intervals (CI) were based on overall survival (OS). Subsequently, the expression of NRGs was combined with survival information of HNSCC in TCGA, the significant prognostic risk factors based on the NRGs were established with the multivariate Cox regression analysis, and the risk score of every patient with HNSCC was calculated by using the following formula: Risk score= $\sum_{i}^{n}=1 Coef_i \times X_i$, $Coef_i$ and X_i represent the risk regression coefficient of NRGs and each NRGs the expression level, respectively. All HNSCC patients were divided into high- and low-risk groups according to the median risk score.

The Kaplan-Meier survival curves, the risk curve and the gene expression heat map were established to analyze whether there is a difference in the OS of HNSCC patients between the low- and high-risk groups. We used the timeROC R package to draw 1-, 3-, and 5-year receiver operating characteristic (ROC) curve of HNSCC patients, the "RMS" and "Survival" package to generate the prognostic nomogram based on age, gender, tumor stage, T stage, N stage, and risk score. Through clinicopathological analysis, the correlation between necroptosis-related prognostic model and other clinical parameters was explored. Univariate Cox and multivariate Cox regression analyses were performed to evaluate whether the prognostic model and other clinical parameters were independent prognostic indicators for patients with HNSCC, and the results were visualized with two forest maps. Decision curve, nomogram and several ROC curves were created, calculating the area under the ROC curve (AUC) and the net benefit of each threshold to compare the predictive ability of those different factors.

Immune cell infiltration analysis

In this study, single-sample GSEA (ssGSEA) was used to explore the different infiltration degrees of immune cell types and the immune-related functions in high- and low-risk groups of the established prognostic model, quantify their relative content via the “GSVA” package. The scores of immune cells and functions in different groups are shown on multi-box plots, respectively.

Prediction of small molecular-targeted drugs against NRGs prognostic model

We divided DE-NRGs into up-regulated and down-regulated groups, uploaded them to the L1000FWD website (https://maayanlab.cloud/L1000FWD/) for obtaining potential small molecular targeted drugs against necroptosis-related prognostic model.

Cell cultivation

Head and neck squamous cell carcinoma cell line, SCC-15, was cultivated in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humid incubator containing 5% CO_2 at 37°C.

Cell proliferation experiment

SCC-15 cells were seeded in the 96-well plate (3000 cells/well) and cultivated in the humid incubator of 37°C and 5% CO_2. According to the experimental requirements, each well was added 10 μL of Cell Counting Kit-8 (CCK-8) solution at 24 h, 48 h, and 72 h respectively, incubating in the dark for 2 h. Then we used the enzyme marker to measure the Optical density (OD) of each well at 450 nm and calculate the cell survival rate (cell survival rate = [(experimental wells OD - blank wells OD)/(control wells OD - blank wells OD)] × 100%).

Statistical analysis

The data analysis of OS time and plot survival curves

was computed using the log-rank test through the R programming language (version 4.1.2). P-value < 0.05 was identified to indicate statistical significance. NRGs significantly correlated with survival were selected using univariate and multivariate Cox analyses. The predictive ability of the risk model was testified using ROC and area under curve (AUC) analyses. Decision Curve Analysis (DCA) was used to show that the prognostic model-based risk score was the superior prognostic factor compared to additional indicators. Besides, we also compare immune infiltrating score and immune functions between low- and high-risk groups by ssGSEA.

Ethics statement

Our study did not require ethical approval, because this study followed the policies and guidelines for data access and publication specified by The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). According to the Declaration of Helsinki, all research performed was approved by the Tianjin Stomatological Hospital.

Results

Identification of NRGs in HNSCC patients

A total of 158 NRGs were obtained from the KEGG website, and the gene expression of 133 NRGs was extracted from the RNA-seq of HNSCC patients. According to the criteria of FDR < 0.05 and |log2 (Fold Change) |> 1/2, we screened the differentially expressed NRGs (DE-NRGs) between normal and tumor samples, finally obtained 50 DE-NRGs, including 42 up-regulated NRGs and 8 down-regulated NRGs (Figures 1A and 1B). In addition, this study further showed the expression rule of the above 50 DE-NRGs in normal and tumor samples by boxplot (Figure 1C). Genetic mutation analysis of these DE-NRGs by using the cBioportal database and a total of 14 genes with mutation rate ≥ 2% was retained among all DE-NRGs. "Amplification" and "deep deletion" were the most common forms of genetic mutation (Figure 1D).

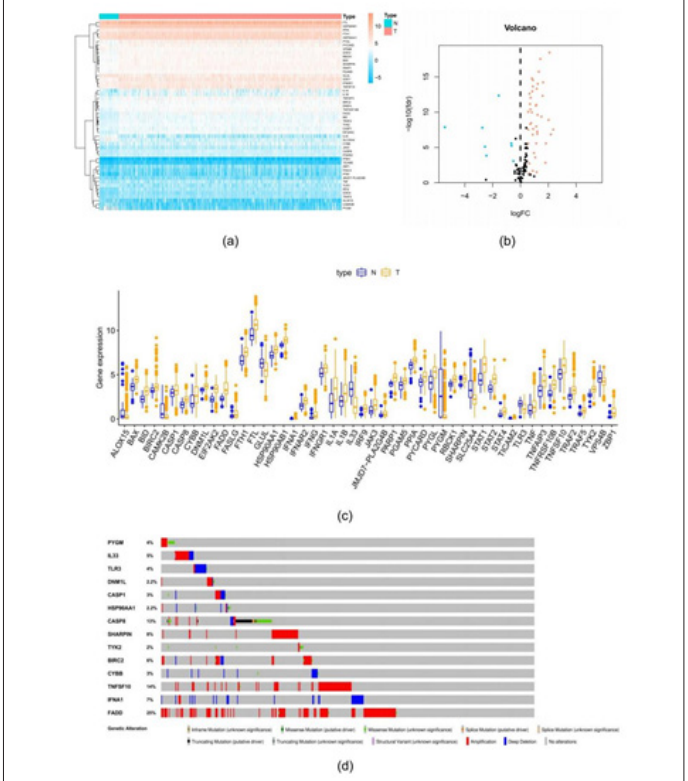

Figure 1. Identification of differentially expressed necroptosis-related genes (DE-NRGs) in patients with HNSCC. (a) A heatmap of the DE-NRGs (FDR < 0.05 and |log2 (Fold Change) |> 1/2). (b) A volcano plot of the DE-NRGs: 42 NRGs with high expression in HNSCC tissue (red), 8 NRGs with low expression in HNSCC tissue (blue), the black color in the volcano plot indicates NRGs with no difference in expression between HNSCC and normal tissues. (c) A boxplot of the DE-NRGs, N is non-tumor tissue, and T is HNSCC tissue. (d) Mutation profile of the DE-NRGs, the results retain the NRGs with a mutation rate ≥ 2%.

GO and KEGG enrichment analyses of DE-NRGs

To investigate the detailed biological functions of these genes, 14 DE-NRGs were conducted into a functional enrichment analysis (Figures 2A, 2B, 2C and 2D). The results of GO enrichment analysis showed that the main biological processes related to DE-NRGs were the NF-kB signaling pathway, cytokine regulation and promotion pathway, and exogenous apoptosis pathway, etc. The cell components are enriched more in cytoplasmic vesicle lumen, vesicle lumen and so on. About molecular functions, DE-NRGs were mainly in cytokine receptor binding, tumor necrosis factor receptor superfamily binding, ubiquitin protein ligase binding, tumor necrosis factor receptor binding and so on. The results of KEGG enrichment analysis showed that DE-NRGs were mainly involved in the pathway: NOD-like receptor signaling pathway signaling pathway, involving necrotic diseases such as influenza A virus and herpes simplex virus type I.

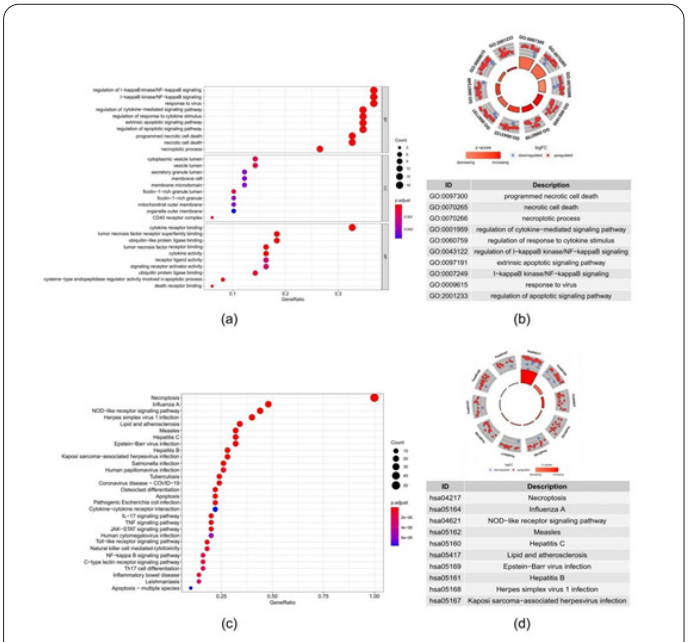

Figure 2. Gene ontology (GO) and Kyoto Encyclopedia of Genes (KEGG) enrichment analyses of differentially expressed necroptosis-related genes (DE-NRGs). (a,b) Bubble and circle plots about the GO enrichment of the 14 DE-NRGs. BP indicates biological process, CC indicates cell components, and MF indicates molecular function. (c,d) Bubble plot and circle plot about the KEGG enrichment of the 14 DE-NRGs.

Establishment of the NRGs prognostic risk model

Using a univariate COX regression analysis, we obtained 12 NRGs which were correlated with the prognosis of HNSCC (P < 0.05) and made a forest plot (Figure 3A). These NRGs were divided into high-risk genes (hazard

ratio (HR) > 1) and low-risk genes (HR < 1). Combined with the survival information of HNSCC patients in TCGA, we constructed a necroptosis-related prognostic risk model composed of 4 NRGs (FTH1, HSP90AA1, IL1A, and TYK2) via multivariate COX regression analysis. Subsequently, the risk score of every HNSCC patient was calculated based on correlation coefficients by using the following formula involving the 4genes: risk score = (0.001391 * FTH1) + (0.001084 * HSP90AA1) + (0.002449 * IL1A) +(-0.06849 * TYK2), and the patients were divided into high- and low-risk groups according to the median value of risk score (1.056027904).

The results of the survival curve showed that the OS time of patients in the high-risk group was significantly lower than that in the low-risk group (P < 0.001) (Figure 3B). To assess the predictive ability of the prognostic risk model, we performed a time-dependent ROC curve analysis. The 1-year, 3-year, and 5-year AUC values of the prognostic model were 0.699, 0.751, and 0.72, respectively (Figure 3C), indicating that the prognostic risk model had high accuracy in predicting the prognosis of HNSCC patients.

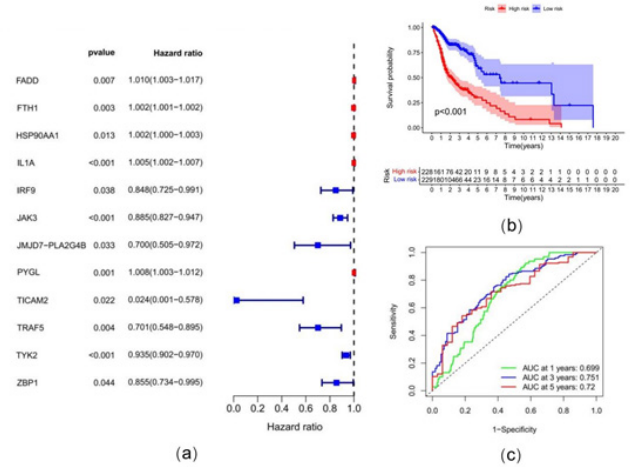

Figure 3. Establishing a necroptosis-related prognostic model. (a) A forest plot of NRGs was obtained by univariate regression analysis, red are high-risk NRGs, and blue are low-risk NRGs. (b) Kaplan-Meier analysis of different OS times between the high-risk group and the low-risk group. (c) Receiver operating characteristic curve analysis at 1-, 3- and 5-year survival rate.

Correlation analysis between the risk model and clinical parameters

Our study further explored the correlation between the prognostic risk model and other clinical parameters of HNSCC patients. All patients were ranked according to the value of risk score and the survival status of patients needed to be analyzed, the result showed that patients in the high-risk group had a worse survival status and more deaths (Figures 4A and 4B). As shown in Figure 4C, the expressions of FTH1, HSP90AA1 and IL1A were significantly increased in the high-risk group compared with the low-risk group, and the expression of TYK2 was significantly decreased in the high-risk group, these results suggest that the four NRGs are related to the progression of HNSCC and the survival status of patients. The correlation between the NRGs prognostic model and other clinical parameters was further analyzed. Among clinical parameters, the risk score was significantly related to the T stage (P < 0.01), tumor stage (P < 0.05) and grade (P < 0.001) (Figure 4D). As there was unknown most of the tumor M stage data, the correlation between the M stage and the risk prognostic model cannot be analyzed.

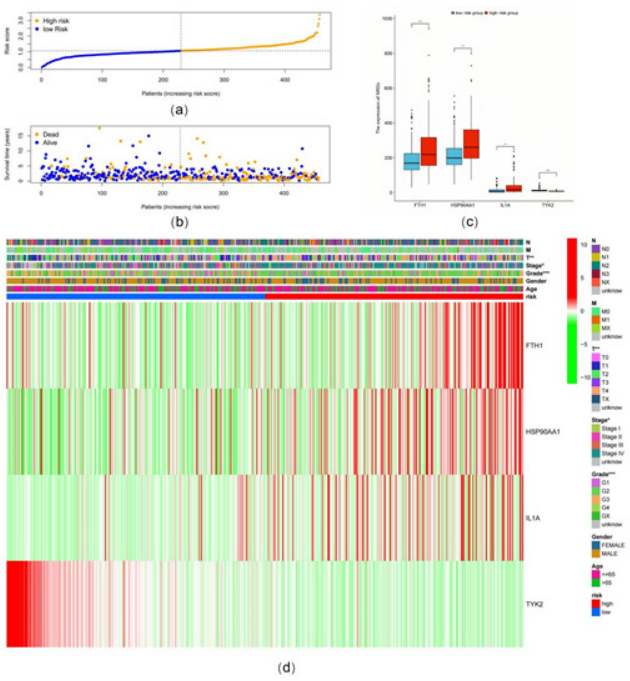

Figure 4. Correlation between necroptosis-related prognostic model and clinical parameters of HNSCC patients. (a) Risk scores of each HNSCC patient. (b) Survival status each of HNSCC patient. (c) A boxplot showing the expression of 4 NRGs in high and low-risk groups, the color blue indicates the low-risk group, and the color red indicates the high-risk group. (d) A heat map of the correlation between risk scores and other clinical parameters. *P < 0.05, **P < 0.01, ***P < 0.001.

An independent prognostic model for HNSCC

Since verified the prognostic model had accurate predictivity, we needed to explore whether this predictive model is an independent prognostic factor for HNSCC. The univariate Cox regression analysis showed that stage, T stage, N stage, and risk score were significantly associated with the survival status of HNSCC patients (Figure 5A). The multivariate Cox regression analysis showed that risk score (hazard ratio = 3.466, confidence interval = 2.421-4.963, P < 0.001) was an independent predictor of survival status in HNSCC patients (Figure 5B).

The prognostic risk model was compared with other clinical characteristics by multivariate ROC analysis and the AUC of the risk score was 0.699, which was greater than that of other clinicopathological variables, indicating that the prognostic risk model established in our study had higher accuracy in predicting prognosis of HNSCC patients than other clinical parameters (Figure 5C).

Then, we used the AUC of the risk score combined TN stage to validate the risk score could be the beneficial supplement in the TN stage (Figure 5D). Decision curve analysis also showed that the risk score curve was farther from the All curve than other clinical parameters, and the risk score combined with the TN stage curve was the farthest from the All curve (Figure 5E). These results implied that the necroptosis-related prognostic risk model of this study as an independent prognostic factor for patients and it also could be used as a useful supplement to predict prognosis based on the TN stage. Therefore, we combined all clinical

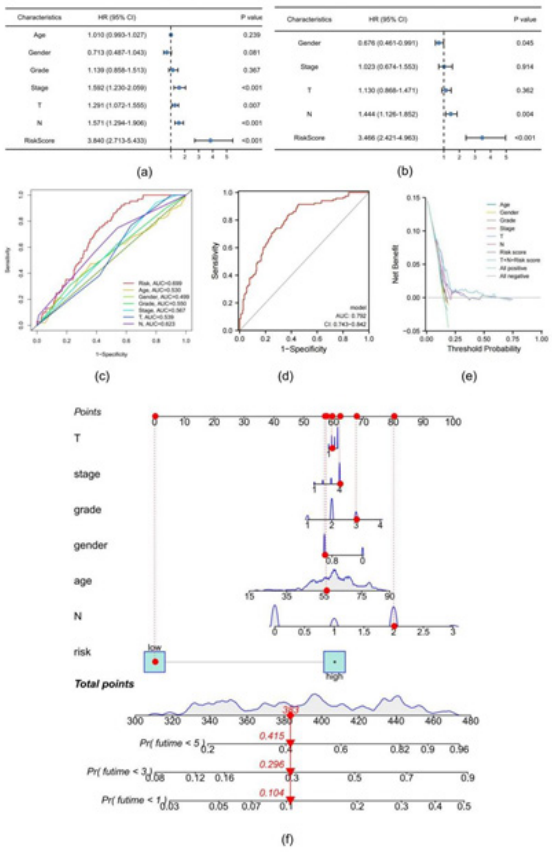

Figure 5. Validation of the predictive ability of the prognostic risk model constructed by necroptosis-related genes (NRGs). (a)Univariate COX regression analysis of clinical characteristics and risk score. (b) Multivariate COX regression analysis of clinical characteristics and risk score. (c) Comparison of the prediction accuracy of the risk model with clinicopathological features, such as age, gender, stage, T stage and N stage. (d) Receiver operating characteristic curve combined analysis of risk score, T stage and N stage. (e) Decision Curve Analysis of each group. (f) A nomogram based on risk score and other clinical parameters.

parameters (gender, age, stage, T stage, N stage, grade) and risk score to construct a nomogram, which was used to accurately predict patient survival rates (Figure 5F).

External validation of the prognostic risk model

The same risk score formula was used to calculate the risk score for each patient in the GSE27020 in the GEO data sets. Patients were also divided into high- and low-risk groups according to the median value of risk score. There were also significant differences in survival status and OS time between the two risk groups ($P < 0.001$). Obtaining the number of patients in the high- and low-risk groups at each time in different groups, the OS time of the patients in the low-risk group was significantly longer than the patients in the high-risk group (Figure 6A). The ROC curve analysis results showed that patients at 1-, 3-, and 5-year AUC were all greater than 0.7 (Figure 6B), which verified that those NRGs constituted the prognostic risk model were promising biomarkers for indicating the prognosis of HNSCC again.

Correlation between risk model and immune cells

To further explore the correlation between risk scores and immune microenvironment, we quantified the enrichment scores of ssGSEA for different immune cell infiltration and function analyses. The results of immune cell

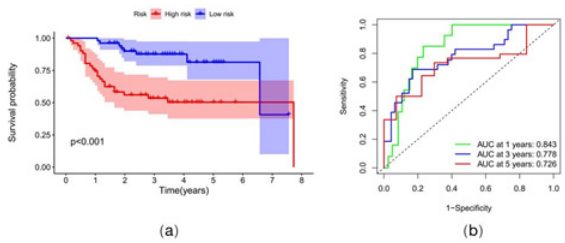

Figure 6. External validation of the prognostic model. (a) Patients in the low-risk group experienced a longer survival time tested by the Kaplan-Meier test. (b) Receiver operating characteristic curve (ROC) analysis based on the GSE27020 data sets.

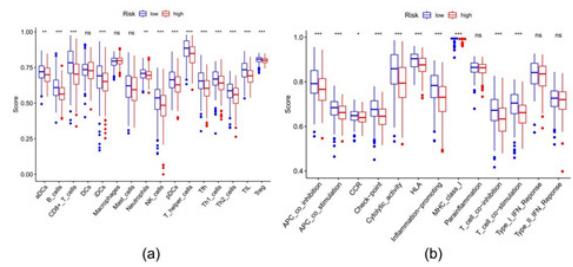

Figure 7. Immune infiltration and function analyses of NRGs prognostic model. (a) Difference analysis of immune cell infiltration between high- and low-risk groups. (b) Difference analysis of immune function between high- and low-risk groups.

infiltration differential analysis and functional differential analysis showed that there were 13 tumor-infiltrating immunocytes (TICs) (activated dendritic cells (aDCs), B cells, CD8+ T cells, immature dendritic cells (iDCs), Neutrophils, NK cells, pDCs, T helper cells, T follicular helper (Tfh) cells, Th1 cells, Th2 cells, tumor-infiltrating lymphocyte (TIL), T regulatory cells (Tregs)) were significantly negatively correlated with the risk score (Figure 7A). In addition, different immune functions also existed between high- and low-risk groups. Except for Para-inflammation, Type I IFN Response and Type II IFN Response, the rest of the immune functions were significantly weakened in the high-risk group (Figure 7B). These findings suggested that, in the low-risk group, the immunological function is more active and might be more sensitive to immunotherapy. So in this study, we further confirmed that the prognostic model is associated with immune activity in HNSCC patients, and those NRGs may be the potential markers of immune status.

The prediction of drugs targeting NRGs

We searched for potential targeted drugs for prognostic models by uploading up-regulated and down-regulated NRGs to the L1000FWD database and obtained the top ten drug candidates shown in Table 1. Among these small-molecular drugs, we selected Mometasone Furoate (MF) and verified it through in vitro experiments.

The inhibited function of MF in the proliferation of SCC-15 cells

In this study, we selected three different concentrations (10 μM, 20 μM, 50 μM) of MF to co-cultivate with SCC-15 cells. The OD of each well was detected after 24 h, 48 h and 72 h, and then the cell survival rate of each group was calculated.

The results showed that the survival rate of SCC-15 cells was significantly decreased in all three MF groups compared with the control group ($P < 0.05$). Interestingly,

Table 1. The top 10 potential drugs found by the L1000FWD database.

Drug	similarity score	P-value	q-value	Z-score	combined score
Radicicol	-0.1702	2.22e-06	5.93e-03	1.67	-9.42
Beclometasone	-0.1489	2.25e-05	2.40e-02	1.68	-7.83
Mometasone Furoate	-0.1489	2.75e-05	2.56e-02	1.81	-8.25
Dinoprostone	-0.1277	1.37e-04	6.56e-02	1.89	-7.31
BRD-A09984573	-0.1277	2.21e-04	6.56e-02	1.71	-6.25
BRD-K66902379	-0.1277	1.55e-04	6.56e-02	1.71	-6.52
BRD-K20914082	-0.1277	2.09e-04	6.56e-02	1.76	-6.47
GP-42	-0.1277	8.51e-05	6.56e-02	1.81	-7.36
L-690330	-0.1277	2.47e-04	6.61e-02	1.75	-6.31
BRD-K40566034	-0.1277	1.86e-04	6.56e-02	1.67	-6.25

at 72 h, the cell viability of each group increased to varying degrees, and even so, it was significantly lower than the control group. Above all, the results indicated that MF inhibited cell proliferation in the dose-dependent approach (Figure 8).

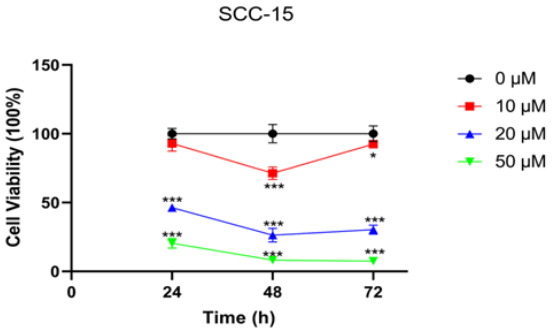

Figure 8. Effects of different concentrations of Mometasone Furoate on the proliferation of SCC-15 cells after 24, 48, and 72 hours, respectively. *P < 0.05, **P < 0.01, ***P < 0.001, compared with 0 μM; n=3.

Discussion

HNSCC is considered to be a malignant tumor. About 35% to 55% of HNSCC patients develop local-regional recurrence or distant metastasis even with intensive multimodality treatment and it also the risk of developing a second primary tumor (13). The recurrence rate of HNSCC has been related to many factors, including tumor location, stage, and human papillomavirus (HPV) infection, as well as the diet (14). The high recurrence rate after anti-tumoral therapies might depend on the molecular and immunological makeup as well as the heterogeneity of HNSCC lesions with unstable genetic drivers and lack of specific biomarkers.

An increased understanding of specific gene signatures represents the basis and rationale for the development and design of novel individualized immunotherapies for cancer patients in the future (15). Therefore, it is of great significance to construct prognostic risk and immunocompetent models based on reliable genes in HNSCC. Current studies suggest that necroptosis could develop an inflammatory tumor immune microenvironment (TIME) by releasing DAMPs, cytokines, and/or chemokines in the tumor microenvironment, resulting in tumor-promoting or anti-tumor effects (16,17). Herein we established the necroptosis-related prognostic model to explore the prognosis and TIME of HNSCC patients.

In the necroptosis-related prognostic model, the higher the risk score, the worse the prognosis of HNSCC patients. We also compared this model with other clinical parameters, the accuracy of our model in predicting OS time of HNSCC patients was higher. The prognostic model includes a total of 4 NRGs (FTH1, HSP90AA1, IL1A and TYK2), which are closely related to tumors and have their biological characteristics. Ferritin heavy chain polypeptide 1 (FTH1) belongs to the ferritin complex and has been reported to participate in cell proliferation and immune response, FTH1 also can regulate the balance of iron ions. Studies have shown that FTH1 can inhibit ferroptosis and promote the occurrence and development of various cancers like liver cancer and bladder cancer (18,19). The polymorphism of the IL1A gene has been demonstrated to enhance the susceptibility of nasopharyngeal carcinoma and IL1A is an important factor of the increased risk in thyroid carcinoma (20,21). Heat Shock Proteins (HSPs) constitute a group of proteins that play a crucial role in protecting cells from chemotherapy, hypoxia and other stress damage. The expression of HSPs was elucidated as a credible hallmark in some cancers, such as breast, prostate, colorectal, lung, ovarian, gastric, oral and esophageal cancer (22). It has been confirmed that HSP90AB1 was highly expressed in HNSCC and associated with T grade, lymph node metastasis, and prognosis (23). In biopsy specimens of tumor tissue, the deletion of HSP90AA1 could be used as a biological marker of favourable prognosis (24). Combining a theoretical/cell culture study with a case-control study, the up-regulation of HSP90AA1 could promote HNSCC lymph node metastasis (25). Genomic and proteomic screens have proved tyrosine kinase 2 (TYK2) as an oncogene promoting progression and metastases of many types of carcinomas, sarcomas, and hematologic cancers (26). TYK2 is a Janus kinase (JAK) that acts as an intermediary between cytokine receptors and STAT transcription factors. Their expression profile in several tumors elucidates that TYK2 signals could stimulate the proliferation, invasion and metastasis of prostate cancer cells (27). In our study, these four NRGs manifested explicit correlations with the prognosis of HNSCC and were utilized to construct a prognostic risk model. Their specific roles and related signal transduction pathways in the occurrence and development of HNSCC still need further study.

The HNSCC, long supposed to be an immunosuppressive disease, and the patients often present with low

absolute number of lymphocytes, spontaneous apoptosis of cytotoxic T lymphocytes, deficiency of natural killer cell (NK) cell activity, and dysfunction of antigen-presenting function (28). The complexity of the immune system is affected by the infiltrating components of immune cells in different patients. Although immunotherapies have begun to evolve as an attractive approach, the benefits of these immunotherapies are not observed in all patients because of the variability in the patients. In the study, we built a necroptosis-related risk model and tried to verify the model's predictive power. Patients were grouped into low- and high-risk groups by the risk model and some analyses such as Kaplan–Meier analysis, ROC analysis, GSEA, and MF prediction. We found risk groups could be a guide in predicting prognoses and researched the correlation between the risk scores and the infiltration as well as functions of immune cells within the TME in HNSCC. In the prognostic model, risk scores were inversely correlated with 13 tumor-infiltrating immunocytes. Among these, NK cells are important immune cells in the body, which are related to anti-tumor, anti-viral infection and immune regulation. CD8+ T cells are the most important immune cells to target and kill cancer cells. During cancer progression, immunosuppressive factors in the TME promote cancer cell growth and metastasis through the weakening or loss of CD8+ T cell function (29). In addition, cancer cells can also change the behavior of neutrophils by releasing cytokines and metalloproteinases, enhancing the chemotaxis of neutrophils and the ability to inhibit apoptosis. Recent studies have shown that the higher neutrophil-to-lymphocyte ratio has a more significant adverse effect on the prognosis of oral cavity cancer (30). These results all indicate that the high-risk group of patients have a lower level of immune infiltrates and weaker immunologic activity compared with the low-risk patients. At the same time, we also detected differences of immune functions between high- and low-risk groups. ssGSEA showed that immune functions about T cell co-stimulation/co-inhibition, APC co-inhibition/co-stimulation, HLA, major histocompatibility complex (MHC) class I molecules, cytolytic activity, and checkpoint were significantly active in the low-risk group. It's worth noting that researchers had investigated that decreased expression of MHC in cancer cells could help tumors evade immune surveillance mechanisms (31). The number of predicted MHC Class I-associated neoantigens was correlated with cytolytic activity and was lower than expected in tumors, suggesting immune-mediated elimination (32).

Tumors responding to immune checkpoint inhibitors (ICIs) have a higher level of immune infiltrates and/or an Interferon (IFN) signature indicative of a T-cell-inflamed phenotype (33). Our findings demonstrated that most of the immune infiltration was elevated in low-risk HNSCC patients compared to the high-risk group. Pembrolizumab and nivolumab are immune checkpoint inhibitors targeting PD-1 that have recently been approved in pretreated recurrent/metastatic (RM)-HNSCC patients (34). Therefore, this signature implied that it would be more advantageous for HNSCC patients at a lower risk to receive immunotherapy.

Our study not only established an effective necroptosis-related prognostic and immune activity model but also screened several small molecular targeted drugs through the database analyzing the up- and down-regulated expression of NRGs. It provides a new idea for the development of effective prognostic markers and immune-targeted drugs in HNSCC patients.

However, this study still has several limitations. The data of this study were derived from the RNA sequencing results of the TCGA database, the prognostic model lacks the validation of multi-center samples and clinical patients' data. In brief, the necroptosis-related prognostic model is expected to be a novel biomarker for the diagnosis and treatment in HNSCC. However, further in vitro and in vivo studies are needed to clarify the role and related mechanisms of NRGs in HNSCC.

This study showed that FTH1, HSP90AA1, IL1A and TYK2 are all over-expressed in HNSCC, and their over-expression is associated with poor prognosis. The prognostic model based on FTH1, HSP90AA1, IL1A and TYK2 can not only effectively predict the prognosis of HNSCC patients but also predict the immune activity. The nomogram established on this basis provides a new idea and direction for the personalized treatment of HNSCC patients.

Availability of data and materials

The data we used to establish the novel risk model in this research are accessible by the public databases: The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) database (accession numbers: GES27020)

Funding

No funding was received.

Conflict of interests

The authors declared no conflict of interest.

References

1. Wan W, Sun B, Yang S, Zhou C, Li Y. Expression of LATS1 in head and neck squamous cell carcinoma and its effect on tumor cell proliferation and invasion. Cell Mol Biol 2022; 68(5): 135-140.
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. Wang J, Liu C, Wei M. Mechanism of Astrin in head and neck squamous cell carcinoma. Cell Mol Biol 2022; 68(7): 141-147.
4. Weinlich R, Oberst A, Beere HM, Green DR. Necroptosis in development, inflammation and disease. Nat Rev Mol Cell Bio 2017; 18(2): 127-136.
5. Snyder AG, Hubbard NW, Messmer MN, et al. Intratumoral activation of the necroptotic pathway components RIPK1 and RIPK3 potentiates antitumor immunity. Sci Immunol 2019; 4(36): eaaw2004.
6. Silke J, Rickard JA, Gerlic M. The diverse role of RIP kinases in necroptosis and inflammation. Nat Immunol 2015; 16(7): 689-697.
7. Liu X, Lieberman J. A Mechanistic Understanding of Pyroptosis: The Fiery Death Triggered by Invasive Infection. Adv Immunol 2017; 135: 81-117.
8. Negroni A, Colantoni E, Cucchiara S, Stronati L. Necroptosis in Intestinal Inflammation and Cancer: New Concepts and Therapeutic Perspectives. Biomolecules 2020; 10(10): 1431.
9. Foria V, Surendra T, Poller DN. Prognostic relevance of extensive necrosis in renal cell carcinoma. J Clin Pathol 2005; 58(1): 39-43.

10. Ando Y, Ohuchida K, Otsubo Y, et al. Necroptosis in pancreatic cancer promotes cancer cell migration and invasion by release of CXCL5. Plos One 2020; 15(1): e228015.
11. Li J, Huang S, Zeng L, et al. Necroptosis in head and neck squamous cell carcinoma: characterization of clinicopathological relevance and in vitro cell model. Cell Death Dis 2020; 11(5): 391.
12. Zhang Z, Hu X, Qiu D, Sun Y, Lei L. Development and Validation of a Necroptosis-Related Prognostic Model in Head and Neck Squamous Cell Carcinoma. J Oncol 2022; 2022: 8402568.
13. Tahmasebi E, Ardestani AK, Madihi N, et al. Evaluation of the current microRNAs expression levels as potential biomarkers in Oral Squamous Cell Carcinoma. Cell Mol Biol 2022; 68(10): 193-198.
14. Peterson LA, Bellile EL, Wolf GT, et al. Cigarette use, comorbidities, and prognosis in a prospective head and neck squamous cell carcinoma population. Head Neck-J Sci Spec 2016; 38(12): 1810-1820.
15. Merlano MC, Denaro N, Garrone O. Immune escape mechanisms in head and neck squamous cell carcinoma and implication for new immunotherapy approach. Curr Opin Oncol 2020; 32(3): 203-209.
16. Raposo TP, Beirao BC, Pang LY, Queiroga FL, Argyle DJ. Inflammation and cancer: till death tears them apart. Vet J 2015; 205(2): 161-174.
17. Sprooten J, De Wijngaert P, Vanmeerbeerk I, et al. Necroptosis in Immuno-Oncology and Cancer Immunotherapy. Cells-Basel 2020; 9(8): 1823.
18. Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. P Natl Acad Sci Usa 2016; 113(34): E4966-E4975.
19. Lin PL, Tang HH, Wu SY, Shaw NS, Su CL. Saponin Formosanin C-induced Ferritinophagy and Ferroptosis in Human Hepatocellular Carcinoma Cells. Antioxidants-Basel 2020; 9(8): 682.
20. Li H, Duan N, Zhang Q, Shao Y. IL1A & IL1B genetic polymorphisms are risk factors for thyroid cancer in a Chinese Han population. Int Immunopharmacol 2019; 76: 105869.
21. Yang ZH, Dai Q, Zhong L, Zhang X, Guo QX, Li SN. Association of IL-1 polymorphisms and IL-1 serum levels with susceptibility to nasopharyngeal carcinoma. Mol Carcinogen 2011; 50(3): 208-214.
22. Saini J, Sharma PK. Clinical, Prognostic and Therapeutic Significance of Heat Shock Proteins in Cancer. Curr Drug Targets 2018; 19(13): 1478-1490.
23. Zhang H, Yin X, Zhang X, et al. HSP90AB1 Promotes the Proliferation, Migration, and Glycolysis of Head and Neck Squamous Cell Carcinoma. Technol Cancer Res T 2022; 21: 2081107206.
24. Buffart TE, Carvalho B, van Grieken NC, et al. Losses of chromosome 5q and 14q are associated with favorable clinical outcome of patients with gastric cancer. Oncologist 2012; 17(5): 653-662.
25. Santos EM, Fraga C, Xavier A, et al. Prion protein is associated with a worse prognosis of head and neck squamous cell carcinoma. J Oral Pathol Med 2021; 50(10): 985-994.
26. Borcherding DC, He K, Amin NV, Hirbe AC. TYK2 in Cancer Metastases: Genomic and Proteomic Discovery. Cancers 2021; 13(16): 4171.
27. Ide H, Nakagawa T, Terado Y, Kamiyama Y, Muto S, Horie S. Tyk2 expression and its signaling enhances the invasiveness of prostate cancer cells. Biochem Bioph Res Co 2008; 369(2): 292-296.
28. Ferris RL, Whiteside TL, Ferrone S. Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. Clin Cancer Res 2006; 12(13): 3890-3895.
29. Wang T, Shen Y, Luyten S, Yang Y, Jiang X. Tissue-resident memory CD8(+) T cells in cancer immunology and immunotherapy. Pharmacol Res 2020; 159: 104876.
30. Tsai YD, Wang CP, Chen CY, et al. Pretreatment circulating monocyte count associated with poor prognosis in patients with oral cavity cancer. Head Neck-J Sci Spec 2014; 36(7): 947-953.
31. Sharpe JC, Abel PD, Gilbertson JA, Brawn P, Foster CS. Modulated expression of human leucocyte antigen class I and class II determinants in hyperplastic and malignant human prostatic epithelium. Br J Urol 1994; 74(5): 609-616.
32. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 2015; 160(1-2): 48-61.
33. Maleki VS. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. J Immunother Cancer 2018; 6(1): 157.
34. Saada-Bouzid E, Defaucheux C, Karabajakian A, et al. Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. Ann Oncol 2017; 28(7): 1605-1611.