Development of a Prognostic Pyroptosis-Related Gene Signature for Head and Neck Squamous Cell Carcinoma Patient

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Abstract

Objective: Head and neck squamous cell carcinoma (HNSCC) is a major threat to public health. Pyroptosis is a form of inflammatory programmed cell death that is still incompletely understood. The role of pyroptotic cell death in HNSCC remains to be fully defined. As such, the present study was developed to explore the potential prognostic utility of a pyroptosis-related gene (PRG) signature in HNSCC.

Methods: PRG expression patterns and the associated mutational landscape in HNSCC were analyzed, after which a 6-gene prognostic model was constructed through least absolute shrinkage and selection operator (LASSO) and Cox regression analyses using the TCGA dataset, followed by validation with two GEO datasets (GSE41643 and GSE65858). Potential predictors of patient outcomes associated with this 6-gene model were identified through topological degree analyses of a protein-protein interaction network. Lastly, the prognostic value of NLRP3 as a predictor of HNSCC patient prognosis was established through immunohistochemical (IHC) analyses of samples from 176 HNSCC patients.

Results: Differentially expressed PRGs were able to readily differentiate between HNSCC tumors and normal tissues. Risk scores derived from the 6-gene PRG model were independent predictors of HNSCC patient prognosis, and genes that were differentially expressed between low- and high-risk groups were associated with tumor immunity. IHC analyses further supported the value of NLRP3 as a predictor of HNSCC patient outcomes.

Conclusions: Overall, these results highlight a novel prognostic gene signature that offers value in the context of HNSCC patient evaluation, although additional research will be essential to elucidate the mechanisms linking these PRGs to HNSCC outcomes.

Introduction

Head and neck cancer is the sixth most prevalent form of cancer globally, with an estimated 830,000 diagnoses and 379,000 deaths occurring annually (1, 2). Over 90% of these cases are diagnosed as head and neck squamous cell carcinoma (HNSCC), which arises from the mucosal epithelium of the oral cavity, pharynx, and larynx(3, 4). Primary treatments for HNSCC patients include various combinations of surgery, radiotherapy, and chemotherapy depending on disease staging (5). As there are no reliable approaches to systematically screening for this disease, a large percentage of HNSCC patients are diagnosed with advanced disease (6). While there have been recent advances in treatment strategies for these patients, their 5-year survival rates remain low, underscoring the importance of developing new approaches to diagnosing and monitoring HNSCC in an individualized manner.

Biomarkers are specific indicators of particular physiological, pathological, or pharmacological processes that can enable effective patient identification and monitoring (7). The advent of high-throughput sequencing technologies has spurred growing interest in the identification of specific biomarkers capable of guiding the prognostic evaluation and treatment of various cancers (8–10). Many predictive

biomarkers associated with HNSCC have been studied to date and may be of value as predictors of long-term patient prognosis and targeted treatment outcomes (11–13). Most of these biomarkers, however, have yet to be subjected to any robust clinical validation or drug targeting efforts (14). Prognostic biomarker models that are better able to predict HNSCC patient prognosis and treatment outcomes in a reliable manner are thus urgently needed to improve survival outcomes for affected individuals.

Pyroptosis is a distinct form of inflammatory programmed cell death from apoptosis(15). Also known as cellular inflammatory necrosis, pyroptosis results in immunostimulatory molecule release from tumor cells (16). At the molecular level, pyroptosis is associated with distinctive molecular features such as chromatin condensation, DNA fragmentation in the absence of DNA damage, and pore formation (17, 18). Pyroptotic cell death has been suggested to play contrasting roles as an inhibitor or promoter of oncogenic progression, and it is closely correlated with cellular migration and proliferation (19–21). Pyroptosis can be induced in response to specific microRNAs (miRNAs), chemotherapeutic drug treatments, or inflammasome activation, suppressing tumor growth (16). Conversely, pyroptosis-related inflammatory cytokine production can give rise to a microenvironment that is better suited to tumor cell growth(22). Pyroptosis-related gene (PRG) signatures can offer insight into prognostic outcomes across a range of cancer types (23, 24). Treatment with TPL can activate GSDME-mediated pyroptosis by inhibiting the expression of HK-II in the mitochondria in HNSCC(25). The prognostic value of PRG signatures in HNSCC, however, remains to be defined.

Herein, we compared PRG expression between control and HNSCC tumor tissue samples to establish the value of these genes as predictors of patient outcomes. A novel PRG-based prognostic model for HNSCC was then developed and validated through both bioinformatics analyses of extant datasets and additional clinical samples.

Materials And Methods

Data processing

RNA-sequencing (RNA-seq) data and matching clinicopathological findings pertaining to 506 patients with HSCC were downloaded from The Cancer Genome Atlas (TCGA) on Aug 10, 2021 (Supplementary Table S1). Somatic and copy number variation (CNV) data were downloaded from the TCGA and UCSC Xena websites. RNA-seq and clinical data used for external validation were downloaded from the GEO database (ID: GSE41643 and GSE65858). R (v 4.0.1) and the R Bioconductor packages were used for all analyses.

Differentially expressed PRG identification

In total, the 33 PRGs listed in Supplementary Table S2 were identified based on prior reviews (16, 26). Given the number of annotated PRGs in TCGA database was 31, the differences in the expression of these genes between HNSCC patients and healthy controls were established using the ‘limma’ package. The Search Tool for the Retrieval of Interacting Genes (STRING) database was further utilized to

construct a protein-protein interaction (PPI) network incorporating these PRGs based on a minimum interaction score of 0.7.

Functional enrichment analyses

The R 'ggplot2' package was utilized to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses.

Prognostic PRG model development

A Cox regression analysis was used to assess the prognostic value of PRGs in HNSCC, with 6 of these significantly prognostic genes being retained for a subsequent prognostic model development through a LASSO Cox regression analysis. HNSCC patients from the TCGA cohort were separated into low- and high-risk groups based upon median risk score value in this cohort, with Kaplan-Meier analyses subsequently being used to compare overall survival (OS) outcomes between these groups. Validation of this model was performed using the GSE41643 and GSE65858 datasets, with risk scores being calculated with the same formula utilized for TCGA patient analyses.

Patients

In total, 176 primary HNSCC patients undergoing surgical treatment at the Department of Oral and Maxillofacial Surgery, the Affiliated Stomatological Hospital of Nanjing Medical University between 2010 and 2015 were recruited for this study WHO classification criteria were used for tumor grading, while UICC and American Joint Commission on Cancer (AJCC) criteria were used for clinical and TNM grading. The Nanjing Medical University Ethics Committee approved this study, which was consistent with the Declaration of Helsinki. All patients provided written informed consent.

Immunohistochemistry (IHC) staining

Paraffin-embedded HNSCC tumor tissue sections (4 µm) were treated with xylene for deparaffinization, rehydrated with an ethanol gradient, treated for 20 min with 3% hydrogen peroxide. Following antigen retrieval, samples were blocked with normal goat serum and then probed overnight with anti-NLRP3 (Proteintech, 19771-1-AP). An HRP-polymer anti-rabbit Kit and a DAB Detection Kit (Fuzhou Maixin Biotech, Fuzhou, China) were then used to stain samples, and hematoxylin was employed for counterstaining. An ethanol gradient was then used to dehydrate samples, which were clarified with xylene and mounted using neutral gum.

Pathological staining analyses

Two pathologists independently analyzed pathological samples. NLRP3 staining was assessed by immunoreactivity score (IRS) values calculated as: $IRS = IS \times PS$. IS scoring was as follow: 0, negative; 1, weak; 2, moderate; 3, strong. PS scoring was as follows: 0, negative; 1, < 10%; 2, 11%-50%; 3, 51%-80%; 4, > 80% positive staining. Patients were then separated into low- and high-expression subgroups based on IRS scores of 0–4 and >4, respectively.

Results

Analysis of the PRG landscape in HNSCC

We began by assessing CNVs evident in 31 annotated PRGs from the TCGA HNSCC dataset, revealing alterations to be present in all of these genes. Of these, the highest CNV amplification frequency was observed for GSDMC, while the highest frequency of deletion was observed for GPX4 (Fig. 1A). The chromosomal locations of these PRGs were next established (Fig. 1B). Of 506 patients in the TCGA HNSCC cohort, 124 (24.51%) harbored somatic mutations in these PRGs, with CASP8 being the most frequently mutated of these genes (Fig. 1C). Missense mutations were the most common (Fig. 1D), with C > T mutations being the most common form of single nucleotide polymorphism (SNP) observed in these patients. PRG expression levels were additionally compared between HNSCC tumors and control tissues, leading to the identification of 21 differentially expressed genes (DEGs) (Fig. 2A), of which 18 (CASP1, CASP3, CASP5, CASP6, CASP8, GSDMB, GSDMD, GSDME, IL-1B, NLRC4, NLRP1, NLRP6, NLRP7, NOD1, PLCG1, PYCARD, SCAF11, and TNF) were upregulated in tumors and three (CASP9, ELANE, and IL-18) were downregulated (Fig. 2B). A PPI network was then constructed to explore interactions among these PRGs in HNSCC (Fig. 2C), and a PRG correlation network was constructed with positive and negative correlations being shown in red and blue, respectively (Fig. 2D).

PRG-based HNSCC patient clustering and functional enrichment analyses

To better understand the relationship between PRG expression and HNSCC, we conducted the consensus clustering of HNSCC patients from the TCGA dataset. Of tested values (2–10), we found that a clustering variable (k) value of 2 was associated with maximal intragroup correlation levels while maintaining low intergroup correlations. As such, these 506 HNSCC patients were effectively separated into two PRG-based clusters (Fig. 3A). When gene expression and clinical profiles were compared between these two clusters (C1 and C2), gender was the only discrepant feature (Fig. 3B). Overall survival (OS) did not differ significantly when comparing patients in these clusters ($P = 0.554$, Fig. 3C).

GO enrichment analyses of these 31 PRGs primarily revealed them to be associated with the positive regulation of cytokine production and interleukin-1 production (Fig. 3D). KEGG enrichment analyses further highlighted a role for these PRGs in the NOD-like receptor signaling and Salmonella infection pathways (Fig. 3E).

PRG-associated prognostic model development

Data pertaining to 500 HNSCC patients for whom survival outcomes were available in the TCGA cohort were used to guide prognostic model development. Initial univariate Cox regression analyses identified 6 PRGs (IL-6, NLRP1, NLRP2, NLRP3, NOD2, and PLCG1) that were significantly associated with patient survival ($P \leq 0.1$), of which IL-6 and NLRP2 were associated with risk increases ($HR > 1$), whereas NLRP1, NLRP3, NOD2, and PLCG1 were protective ($HR < 1$) (Fig. 4A). Next, least absolute shrinkage and selection

operator (LASSO) (Fig. 4B-C) and Cox regression analyses (Fig. 4D) were utilized to conduct a 6-gene model based on an optimal λ value. This model was as follows: risk score = $(0.0871 \times \text{IL6 exp.}) + (0.3764 \times \text{NLRP1 exp.}) + (0.0595 \times \text{NLRP2 exp.}) + (0.3820 \times \text{NLRP3 exp.}) + (0.2357 \times \text{NOD2 exp.}) + (0.1371 \times \text{PLG1 exp.})$. These HNSCC patients were then stratified into low- and high-risk groups based upon median risk score values (Fig. 4E). In a principal component analysis (PCA), low- and high-risk patients were readily separated into two clusters (Fig. 4F). High-risk HNSCC patients exhibited poorer OS outcomes relative to low-risk patients ($P < 0.001$) (Fig. 4G). Time-dependent receiver operating characteristic (ROC) analyses were also utilized to assess the specificity and sensitivity of this model, revealing area under the ROC curve (AUC) values for 1-, 2-, and 3-year survival outcomes of 0.610, 0.654, and 0.703 (Fig. 4H).

External risk signature validation

To validate the risk signature developed above, data pertaining to 367 HNSCC patients from the GSE41643 and GSE65858 datasets were utilized. Batch effects between these datasets were reduced with the 'ComBat' algorithm. These patients were then separated into high-risk ($n = 190$) and low-risk ($n = 177$) groups based upon the median risk scores from the TCGA cohort (Fig. 5A). PCA analysis revealed that these two subgroups were appropriately separated from one another (Fig. 5B). Significant differences in OS were evident between low- and high-risk HNSCC patient groups ($P = 0.002$) (Fig. 5C). ROC curve analyses further confirmed the predictive accuracy of this model, with AUC values pertaining to 1-, 2-, and 3-year survival outcomes of 0.611, 0.601, and 0.558 (Fig. 5D).

Analysis of risk score prognostic value and predictive nomogram construction

Univariate and multivariate analyses were next performed to explore the prognostic utility of the risk score developed above. In univariate analyses, this risk score was able to independently predict survival outcomes in the TCGA and GEO datasets (TCGA, HR = 2.060; GEO, HR = 1.714) (Fig. 6A, D). In multivariate analyses, risk scores were similarly able to independently predict HNSCC patient prognosis (TCGA, HR = 1.977, GEO, HR: 1.910) (Fig. 6B, E). When a heatmap was constructed incorporating clinical findings from the TCGA (Fig. 6C) and GEO (Fig. 6F) cohorts, we observed diverse gender distributions between low- and high-risk patients in GEO cohorts ($P < 0.05$). We then constructed a nomogram incorporating all independent prognostic variables from multivariate regression analyses, and we found that this model was able to effectively predict 1-, 3-, and 5-year OS in both GEO and TCGA cohorts (Fig. 6G-I).

The relationship between prognostic PRGs and immune functionality

Single-sample gene set enrichment analysis (ssGSEA) scores were next used to compare enrichment scores pertaining to 16 immune cell types and 13 immune-related pathways between high- and low-risk

HNSCC patients in the TCGA and GEO validation cohorts used above. High-risk patients were found to exhibit increased iDC infiltration in the TCGA cohort (Fig. 7A), while NK cell infiltration was increase and macrophage infiltration was decreased in high-risk patients from the GSE65858 cohort (Fig. 7B). No significant differences in immune status were evident in the TCGA or GSE41643 cohorts (Fig. 7D, F), while cytolytic activity and inflammation-promoting functionality were enriched among high-risk individuals in the GSE65858 cohort (Fig. 7E).

When correlations between the 6 prognostic PRGs defined above and immune infiltration were assessed with the TIMER database, NLRP3 was found to be the only gene positively correlated with B cell, CD8 + T cell, CD4 + T cell, macrophage, neutrophil, and dendritic cell abundance (Fig. 8).

Lower expression of NLRP3 is related to a poor HNSCC patient prognosis

Topological degree analyses of the 31 PRGs in the PPI network developed above led to the selection of the top 8 of these genes. Of these, only NLRP3 was included in our prognostic gene signature (Fig. 9A). Given the harzard ratio of NLRP3 in the prognostic model was 0.68 ($p = 0.039$). We additionally conducted IHC analyses of NLRP3 staining in samples from 176 patients with HNSCC (Fig. 9B), the results demonstrated that the NLRP3 was significantly correlated with tumor size ($P = 0.023$), however, there was no significant relationship between NLRP3 expression and gender, age, tumor location, distant metastasis, pathological grade, infiltration, clinical stage or recurrence (Supplementary Table S3). In addition, the lower expression level of NLRP3 associated with the poor survival of HNSCC patients (Fig. 9C-D). Together, these findings confirm the prognostic utility of NLRP3 as a predictor of HNSCC patient survival.

Discussion

Normal physiological homeostasis is dependent on maintaining a balance between cellular proliferation, death, and differentiation (16). While apoptosis and necrosis were once believed to be the primary forms of cell death, pyroptosis, autophagy, and other mechanisms have also been shown to mediate a loss of cellular viability (27). Pyroptotic cell death is highly inflammatory and plays a role in a variety of pathogenic processes (28–30). Inflammasome activation initiates pyroptosis by recruiting caspase-1. Gasdermin D (GSDMD) serves as a caspase-1/11 substrate, inducing pyroptotic cell death owing to its ability to promote non-selective pore formation within the plasma membrane, subsequently driving cellular swelling, rupture, and the release of proinflammatory factors such as HMGB1, ATP, and IL-1 β (16, 18, 31). The relationship between pyroptosis and cancer is complex, given that this form of cell death can both drive tumor progression and impair antitumor immunity (18), while also inhibiting oncogenesis (32). The mechanisms whereby PRGs affect HNSCC progression and survival outcomes remain to be clarified. Herein, we evaluated expression levels of 31 different PRGs in samples from HNSCC patients in the TCGA database, revealing 21 of these genes to be differentially expressed in HNSCC including 18 that were upregulated in this oncogenic setting. GO and KEGG analyses demonstrated a link between PRGs and

cytokine production, IL-1 production, NLRP signaling, and Salmonella infection. These functional pathways were closely associated with HNSCC oncogenesis and progression. Salmonella infection, for example, can promote TLR4/ MyD88 pathway activation and consequent increases in macrophage and neutrophil infiltration (33). In OSCC, targeting of the ROS/NLRP3 inflammasome/IL-1 β signaling pathway has been suggested to improve outcomes associated with 5-FU adjuvant chemotherapy (34). These data suggest that in-depth PRG studies may offer improved insight into antitumor immunity and inflammation.

HNSCC patients exhibit poor long-term survival outcomes, underscoring the need for more reliable biomarkers of long-term patient prognosis and treatment outcomes. TNM stage, vascular invasion, and other traditional clinicopathological biomarkers have yielded unsatisfactory outcomes when used to gauge patient prognosis (35). RNA-seq and other high-throughput sequencing technologies have led to the identification of a wide variety of prognostic biomarkers associated with different cancers (36, 37). Recent work has shown individual biomarkers are ill-suited to gauging cancer patient prognosis. A 4-gene immune-related biomarker signature (PVR, TNFRSF12A, IL21R, and SOCS1) may, together with other clinicopathological metrics, offer value as a means of assessing HNSCC patient prognosis (38). Wang et al. assessed patterns of gene expression from 771 HNSCC patients in the TCGA and GEO databases, leading to the development of a 6-gene prognostic risk signature that was able to independently predict patient survival (39). In a similar vein, we herein assessed the prognostic value of PRGs in HNSCC. We ultimately employed a LASSO Cox regression approach to construct a 6-PRG (IL-6, NLRP1, NLRP2, NLRP3, NOD2, and PLCG1) prognostic risk signature. Risk scores derived from this model were able to effectively stratify patients into low- and high-risk cohorts. High-risk patients exhibited worse survival outcomes than did low-risk patients. These findings thus confirm the prognostic value of this novel PRG risk signature, highlighting a novel approach to predicting HNSCC patient outcomes.

Cell death plays a central role in diverse pathological processes (40), with pyroptosis functioning as an inflammatory type of caspase-mediated inflammatory cell death that can modulate the immunogenic potential of specific cancers(41). Such immunogenicity is of critical importance in the context of tumor immunotherapy owing to the ability of tumor cells to activate a variety of immunosuppressive pathways within the local tumor microenvironment (42). Pyroptosis can impact immune cell composition and associated immunological pathway activation to alter the processes governing tumorigenesis. Herein, we compared the immunological status of patients in our low- and high-risk groups, and assessed the relationship between the levels of difference prognostic PRGs in HNSCC (IL-6, NLRP1, NLRP2, NLRP3, NOD2, and PLCG1) and immune cell infiltration. The results of these analyses suggest that this prognostic gene signature may have important implications for immunotherapy treatment planning. Of these genes, only NLRP3 was positively correlated with immune infiltration in the TIMER database. The activation of NLRP3 has previously been reported in the context of metabolic changes, mitochondrial dysfunction, and the disassembly of the Golgi compartment (43). The NLRP3 inflammasome can also promote the caspase-1-dependent release of IL-1 β and IL-18, which are inflammatory cytokines, in addition to activating downstream pyroptotic signaling mechanisms (43). Wang et al. observed a positive correlation between NLRP3 expression levels and lymph node metastasis, tumor size, and IL-1 β levels in

OSCC patients, highlighting this pathway as a promising therapeutic target (44). However, our study showed differences from the previous studies, confirming the dual role of pyroptosis. Based on the cox multivariate regression analysis of NLRP3 in risk model, we noticed that NLRP3 might act as a potential indicator for predicting the HNSCC patients' outcomes (HR = 0.68, p = 0.039). In line with these reports, the results of our PPI network and survival analyses suggested that NLRP3 may be a valuable predictor of prognosis among HNSCC patients. Lower levels of NLRP3 expression detected via IHC staining were associated with a worse prognosis. Overall, these data demonstrate the value of NLRP3 as a PRG that is independently associated with HNSCC patient survival.

Conclusions

In conclusion, the results of this study suggest that many differentially expressed PRGs were closely linked to the pathogenesis of HNSCC. Risk scores derived from our 6-gene PRG model were independent predictors of HNSCC patient outcomes, and genes that were differentially expressed between low- and high-risk groups were associated with tumor immunity. IHC analyses further supported the value of NLRP3 as a predictor of HNSCC patient prognosis. Overall, these data highlight a novel gene signature that can be leveraged to predict survival outcomes in individuals with HNSCC, offering a foundation for future analyses of the link between PRGs and this cancer type.

Abbreviations

Head and neck squamous cell carcinoma

HNSCC

pyroptosis-related genes

PRGs

RNA-sequencing

RNA-seq

The Cancer Genome Atlas

TCGA

Gene Expression Omnibus

GEO

protein-protein interaction

PPI

Search Tool for the Retrieval of Interacting Genes

STRING

Gene Ontology

GO

Biological Process

BP

Cellular Component

CC
Molecular Function
MF
Kyoto Encyclopedia of Genes and Genomes
KEGG
Immunohistochemistry
IHC
Single-Sample Gene Set Enrichment Analysis
ssGSEA.

Declarations

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Authors' contributions

WWZ and JYZ have the same contribution in this work. WWZ and JYZ analyzed the data and wrote paper. LKL applied the funding and designed the experiments. WWZ, MYW and HZ performed the experiments. WWZ, RDZ, MQW, JW and LKL edited the paper, YBX revised the manuscript. All authors contributed to the article and approved the submitted version. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Nanjing Medical University and samples were obtained with written informed consent from all patients, and all informed consent were conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Landscape of the genetic and expression variation of PRGs in HNSCC. (A) The CNV frequency of 31 PRG in the HNSCC cohort. The height of the column represented the CNV frequency. (B) The location of PRGs

on chromosomes in TCGA cohort. (C) The mutation frequency of 31 PRGs in HNSCC cohort. (D) The mutation classification of the 31 PRGs in TCGA cohort.

Figure 2

Expressions of the 31 pyroptosis-related genes and the interactions among them. (A) Heatmap (blue: low expression level; red: high expression level) of the pyroptosis-related genes between the normal (N, brilliant blue) and the tumor tissues (T, red). (B) The expression of 31 PRG in HNSCC and normal tissues, Tumor, red; Normal, blue. The upper and lower ends of the boxes represented the interquartile range of values. The lines in the boxes represented median value. (C) PPI network showing the interactions of the pyroptosis-related genes (interaction score = 0.7). (D) The correlation network of the pyroptosis-related genes (red line: positive correlation; blue line: negative correlation). The depth of the colors reflects the strength of the relevance). P values were showed as: *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 3

Tumor classifications and functional enrichments of PRGs in HNSCC. (A) 506 HNSCC patients were grouped into two clusters according to the consensus clustering matrix ($k = 2$). (B) Heatmap of the clinicopathologic characters of the two clusters classified by these DEGs. (C) Kaplan–Meier OS curves for the two clusters. (D) The enriched item in gene ontology analysis (BP biological process, CC cellular component, MF molecular function.). (E) The enriched item in Kyoto Encyclopedia of Genes and Genomes analysis. P value was showed as: * $P < 0.05$.

Figure 4

Construction of the risk signature in TCGA cohort. (A) Univariate cox regression analysis of OS for each pyroptosis-related gene, and 6 genes with $P \leq 0.1$. (B) LASSO regression of the 6 OS-related genes. (C) Cross-validation for tuning the parameter selection in the LASSO regression. (D) Cox regression analysis for constructing the 6-gene prognostic model. (E) The survival status for each patient (low-risk population: on the left side of the dotted line; high-risk population: on the right side of the dotted line) (Upper panel). Distribution of patients based on the risk score (Lower panel). (F) PCA plot for HNSCC based on the risk score. (G) Kaplan-Meier curves for the OS of patients in the high- and low-risk groups. (H) ROC curves demonstrated the predictive efficiency of the risk score.

Figure 5

External validation of the risk signature in GEO cohort. (A) The survival status for each patient in validation cohort (low-risk population: on the left side of the dotted line; high-risk population: on the right side of the dotted line) (Upper panel). Distribution of patients in the GEO cohort based on the median risk score in the TCGA cohort (Lower panel). (B) PCA plot for HNSCC patients in GEO cohort. (C) Kaplan-Meier curves for comparison of the OS between low- and high-risk groups. (D) Time-dependent ROC curves for HNSCC.

Figure 6

Construction of predictive nomogram and independent prognostic value of risk score. (A-B) Hazard ratio and P-value of the constituents involved in univariate Cox regression analysis and multivariate analysis considering clinical the parameters and six prognostic PRGs in TCGA cohort. (C) Heatmap (blue: low expression; red: high expression) for the connections between clinicopathologic features and the risk groups in TCGA cohort. (D-E) Hazard ratio and P-value of the constituents involved in univariate Cox

regression analysis and multivariate analysis considering clinical the parameters and six prognostic PRGs in GEO cohort. (F) Heatmap for the connections between clinicopathologic features and the risk groups in GEO cohort.. (G-I) Nomogram to predict the 1-year, 3-year, and 5-year overall survival rate of HNSCC patients. Calibration curve for the overall survival nomogram model in the discovery group. A dashed diagonal line represents the ideal nomogram. P values were showed as: *P < 0.05; ***P < 0.001.

Figure 7

Comparison of the ssGSEA scores for immune cells and immune pathways. (A) Comparison of the enrichment scores of 16 types of immune cells between low- (blue box) and high-risk (red box) group in the TCGA cohort. (B-C) Comparison of the enrichment scores of the immune cells between low- and high-risk group in two GEO cohort. (D) Comparison of the enrichment scores of immune-related pathways between low- and high-risk group in the TCGA cohort. (E-F) Comparison of the enrichment scores of immune-related pathways between low- and high-risk group in the GEO cohort.

Figure 8

The association between six prognostic PRGs and immune infiltration (TIMER). The association between the abundance of immune cells and the expression of (A) IL-6, (B) NLRP1, (C) NLRP2, (D) NLRP3, (E) NOD2 and (F) PLCG1 in HNSCC.

Figure 9

NLRP3 is associated with the poor prognosis of HNSCC patients. (A) The top 8 ranked proteins in topological degree in PPI network (Left panel), the venn diagram showed that NLRP3 was the only protein which existed in both PPI network and risk model (Right panel). (B) NLRP3 expression in specimens of HNSCC patients. (n=176). (C) OS curves for HNSCC patients with NLRP3-low and NLRP3-high expression (P=0.019). (D) Kaplan-Meier analysis of DFS curves for HNSCC patients with NLRP3-low and NLRP3-high expression (P=0.267).

Supplementary Files

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