Exploration of a predictive model based on genes associated with fatty acid metabolism and clinical treatment for head and neck squamous cell carcinoma

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Abstract

**Background:** Head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent malignant tumors of the head and neck and presents high risks of recurrence and poor prognosis postoperatively. The aim of this study was to establish a predictive model based on fatty acid metabolism (FAM) genes to forecast the prognosis of HNSCC patients and the subsequent treatment strategies.

**Methods:** We accessed the TCGA and GEO databases for HNSCC genes and clinical data. The FAM risk score model was created and validated using a combination of univariate Cox analysis and least absolute shrinkage and selection operator (LASSO) regression analysis. Combining risk scores and clinical characteristics, a nomogram was established and assessed. Subsequently, the function, gene mutation, immune difference, and chemotherapeutic drug sensitivity of the groups with high- and low-risk scores were analyzed. Consequently, the model's validity was evaluated comprehensively by combining single gene analysis.

**Results:** The FAM risk score model for predicting HNSCC prognosis had certain validity. Patients in the high- and low-risk groups had genetic mutations, and the prognosis was the poorest for the high-risk groups with high genetic mutations. The patients with low-risk scores were suitable for immunotherapy since they had increased infiltration of immune cells. In contrast, the patients in the other groups were more suitable for chemotherapy.

**Conclusion:** The results of this study demonstrated that the FAM risk score model may predict the prognosis of HNSCC and has a certain therapeutic guidance value.

**KEYWORDS**

chemotherapy, fatty acid metabolism, head and neck squamous cell carcinoma, immunotherapy, prognosis
INTRODUCTION

As the most common pathological type of head and neck tumor, head and neck squamous cell carcinoma (HNSCC) has a high incidence.\textsuperscript{1,2} In the literature, the global incidence of HNSCC is high worldwide, accounting for 89,000 newly diagnosed cases and 450,000 deaths in 2018.\textsuperscript{3,4} HNSCC usually occurs in the nasopharynx, sinuses, oropharynx, hypopharynx, larynx, and oral cavity,\textsuperscript{5} and its etiologies include smoking, alcohol consumption, and genetic and viral infections.\textsuperscript{6,7} At present, the combination of surgery, radiotherapy, and chemotherapy is a routine clinical treatment strategy.\textsuperscript{8,9} Unfortunately, the patients’ prognosis is unsatisfied. The postoperative recurrence rate of patients is 40%-60%,\textsuperscript{9} and the five-year survival rate is only approximately 50%.\textsuperscript{10} Moreover, few studies have reported effective indicators to predict the prognosis of HNSCC worldwide. Therefore, it is essential to find reliable strategies to predict the prognosis of HNSCC.

Due to the fast growth and absence of blood arteries, tumor cells usually exist in the acidic, hypoxic, and dystrophic tumor microenvironment.\textsuperscript{11,12} Consequently, tumor cells exhibit notable variations in protein, fatty acid, and glucose metabolism from normal cells.\textsuperscript{13} Tumor cells are revealed to be able to more readily utilize the higher energy provided by fatty acid metabolism (FAM).\textsuperscript{14,15} Genes in the fatty acid metabolic pathway are known as fatty acid metabolism-associated genes (FAMGs), whose reprogramming is an important guarantee for the continuous growth, proliferation, and migration of cancer cells.\textsuperscript{16,17} In particular, FAMGs have potential mechanistic and prognostic roles in HNSCC.\textsuperscript{18} Visweswaran et al. proposed that targeting lipid metabolism may serve as a novel approach for tumor therapy.\textsuperscript{19} Constructing a prognostic risk model is a feasible solution for evaluating tumor prognostic indicators. Currently, Du et al.\textsuperscript{15} have reported a new risk model based on FAM to assess the prognosis of HNSCC. However, the function of FAMGs in HNSCC remains unknown, and systematic studies still need to be established to investigate the role of FAMGs in the prognosis of HNSCC.

In this study, we constructed a predictive risk model for HNSCC based on 20 FAMGs screened in the HNSCC cohort of the TCGA database. We also investigated the relationship between this prognostic risk model and the immune response. The aim of this study was to evaluate the value of the FAM prognostic risk scoring model for the diagnosis and treatment of HNSCC in clinical settings.

METHODS AND MATERIALS

2.1 Data downloading and processing

A total of 548 RNA sequencing data profiles, including 504 HNSCC tumor samples and 44 normal tissue samples, were obtained as fragments per kilobase million from the TCGA–HNSC cohort in the TCGA database. Meanwhile, their clinical characteristics were downloaded, including age, sex, tumor grade, tumor stage, prognostic information, and other relevant information. Similarly, we utilized genetic information of the HNSCC patients collected in the Gene Expression Omnibus (GEO) database as a test set (GSE41613, including 94 samples).

2.2 Construction and validation of the prognostic model

According to the previous studies,\textsuperscript{15,20} we obtained 309 FAMGs to construct this analysis (Appendix S1). The differentially expressed fatty acid metabolism-related genes (DEFAMGs) between normal and HNSCC samples were identified according to the criteria of $|\log_{2} FC| > 0.585$ and false discovery rate $< 0.05$. The prognostic DEFAMGs were determined using univariate Cox regression analysis ($p < 0.05$), and gene mutations in the tumor samples were also analyzed. A prognostic risk score model was created with the assistance of least absolute shrinkage and selection operator (LASSO)
regression analysis, aiming to predict HNSCC patient prognosis. Tenfold cross-validation was performed with over 1000 cycles of analysis. The risk score model was calculated using the following formula: Risk score = Σ coefficient of genes * expression values of genes.

All subjects were divided into high- and low-risk groups according to the median risk scores after each sample’s risk score was calculated with the formula. To confirm the value of the above risk score model to predict patient prognosis, the log-rank test and Kaplan–Meier survival curves were used to compare the overall survival (OS) of the patients in both groups. Subsequently, we drew the receiver operating characteristic (ROC) curve and determined the area under the curve (AUC) values to assess the efficacy of the patient prognostic model. In addition, univariate and multivariate Cox regression analyses were carried out to estimate whether the risk prediction model was an independent predictive factor when compared to the clinical features (e.g., age, sex, grade, and stage).

2.3 Establishment of the nomogram

The nomogram was constructed using the data from the TCGA-HNSCC cohort, including age, tumor stage, and risk score. Furthermore, both calibration curves and ROC curves were drawn to examine the nomogram’s validity and its prognostic value, respectively. In addition, we performed univariate and multivariate Cox regression analyses to judge whether the nomogram can be used as an independent indicator for predicting the prognosis of HNSCC.

2.4 GSVA

To further compare FAM between the high- and low-risk groups, gene set variation analysis (GSVA) based on the gene profiles was conducted. GSVA is a nonparametric and unsupervised approach for calculating gene enrichment, which can be used to evaluate a specific biological process. Statistics were judged significant when \( p < 0.05 \).
FIGURE 5 Functional analysis of DEGs. (A) Heatmap is showing the GSVA of DEGs; (B) Circle plot for GO analysis of DEGs; (C) Bar plot for GO analysis of DEGs; (D) Circle plot for KEGG analysis of DEGs; (E) Bar plot for KEGG analysis of DEGs.
Correlation between the risk score and immune factors

FAM processes have been reported to be associated with immune cells and tumor progression. According to CIBERSORT, which is used to assess immune cell infiltration, we investigated the relationship between the predictive model and immune infiltration in HNSCC using the Wilcoxon signed-rank test.

GO and KEGG

The differentially expressed genes (DEGs) in both the high- and low-risk groups were retrieved by the R package "limma." Moreover, the “clusterProfiler” R package was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. KEGG is capable of identifying the main biological properties and functional pathways of DEGs.

Protein–protein interaction network

We analyzed DEGs through the STRING online database to generate protein–protein interaction (PPI) networks. The PPI network was further processed via Cytoscape software (version 3.7.2). Additionally, we used CytoHubba, a plug-in of Cytoscape, to screen out the hub genes among the DEGs. Based on the level of hub gene expression, the samples were categorized as...
upregulated or downregulated. Finally, we conducted a survival analysis and compared immune cell infiltration.

2.8 Sensitivity analysis of chemotherapy drugs

The pRRophetic software package was used to predict the half-maximal inhibitory concentration (IC50) of chemotherapeutic drugs in each sample, including cisplatin, docetaxel, gemcitabine, and paclitaxel, which are routinely utilized for the treatment of HNSCC. The differences in IC50 between the two groups were compared to evaluate the clinical efficacy of chemotherapy for the treatment of HNSCC.

3 RESULTS

3.1 Establishment and verification of the risk score model

The flow chart of this study is presented in Figure 1. According to the expression of FAMGs, we obtained 129 DEFAMGs from the gene expression matrix that were defined as differential genes. Specifically, the top 50 genes highly expressed in normal and tumor samples were selected. Heatmaps (Figure 2A) and volcano plots (Figure 2B) were drawn to demonstrate DEFAMGs between the normal and tumor samples.
Based on the uni-Cox regression analysis, 22 FAMGs were shown to be substantially linked with patient prognosis (p < 0.05) (Figure 2C). The frequencies of 22 prognostic FAMGs somatic mutation profiles were summarized in Figure 2D, achieving a total of 10.39% in 510 HNSCC samples. In addition, the correlations of these 22 FAMGs were analyzed as well, and the results were shown in Figure 2E.

Similarly, with the assistance of LASSO regression analysis, 20 FAMGs were selected to establish a predictive risk score model (Figure 2F,G). The risk score for each HNSCC patient was calculated as follows: Risk score = −(0.267068370405719 × ACAA1) + (0.163238015569013 × PRKAA2) + (0.082678299650745 × OSTC) + (0.100868138563843 × HSPH1) − (0.117924288651454 × PTGDS) + (0.157079934784576 × PHYH) + (0.0439834205998577 × PTGES3) − (0.435964503575487 × CYP8B1) − (0.694856005960078 × THRSP) − (0.0272691081848022 × ACOX3) − (2.69032042245729 × ACSBG2) + (0.1350935514509 × LDHA) + (0.0861616365857884 × HADHB) − (0.0512350179850526 × ACMS3) + (0.0443599211083752 × SMI) + (0.188524223062724 × ELOVL6) + (0.363348040751783 × ACADL) − (0.263268335672849 × ACAB) + (0.0080137520740822 × HSP90AA1) + (0.0587043322637337 × SLC25A17).

Subsequently, as shown in Figure 3A, patients were divided into low- and high-risk groups based on the median risk score, which indicated that patients can be distinguished clearly by this prognostic model. Referring to the K–M survival analysis, patients in the high-risk group had worse OS than those in the low-risk group (Figure 3C), which was also validated by an external test cohort (Figure 3D).

As shown in Figure 3F, the one-year, three-year, and five-year AUC values on the ROC curves of the risk model were 0.689, 0.712, and 0.719, respectively, revealing much better predictive performance than other features (Figure 3G).

### 3.2 Establishment of the nomogram for OS

The independent prognostic analyses of the risk model mainly included multiple clinical factors, including age, sex, tumor grade, and clinical stage. Combining uni- and multi-Cox regression analyses, age, clinical stage, and risk score were considered three independent prognostic factors (Figure 3H,I). Based on the above results, a nomogram combining age, clinical stage, and risk score was constructed (Figure 4A). For one-year, three-year, and five-year calibration curves (Figure 4B), it is clear that nomograms are beneficial for predicting HNSCC prognosis with high accuracy. The Cox analysis revealed that the nomogram may be considered an independent feature for prognostic prediction (Figure 4C,D).

### 3.3 Functional analysis of DEGs

To explore differential biological functions between the high- and low-risk score groups, GSVA enrichment analysis was conducted, showing that the majority of metabolic pathways (e.g., FAM) were enriched in the high-risk score group (Figure 5A).

Moreover, we performed GO and KEGG analyses for further functional analysis of the DEGs. The DEGs were considerably enriched within the biological process category in the GO circle graph,
FIGURE 9  PPI network and single gene analysis. (A) PPI network; (B) Hub gene network processed by cytoHubaa; (C) Survival probability analysis for SPRR3; (D) Effect of SPRR3 on immune cells
indicating their importance (Figure 5B). The GO bar plot showed that the DEGs were highly enriched in antigen binding among the molecular functions and immunoglobulin complexes among the cellular components. In terms of the biological processes, DEGs were mainly enriched in the regulation of B-cell activation, B-cell receptor signaling pathway, and complement activation (Figure 5C, Appendix S1). KEGG enrichment analysis demonstrated that these genes mainly participated in cytokine–cytokine receptor interactions, Staphylococcus aureus infection, the IL-17 signaling pathway, and viral protein interactions with cytokines and cytokine receptors (Figure 5D,E, Appendix S1).

3.4 | Mutation differences between high- and low-risk groups

The difference in the top 15 mutated genes was compared and visualized in waterfall plots (Figure 6A,B). As the topmost mutant genes, we assessed the differences in risk score between the TP53 wild-type and mutation-type, which suggested that the TP53 mutation-type has a higher risk (Figure 6C). Survival analysis revealed that low tumor mutation burden (TMB) was associated with a more favorable outcome than high TMB. Comprehensive analysis of TMB and risk score factors showed that patients in the low-risk group with low TMB had a favorable prognosis compared to the other categories, including high TMB in the high-risk group, high TMB in the low-risk group, and low TMB in the high-risk group (Figure 6D).

3.5 | Relationship between immune factors and the risk score model

The boxplot (Figure 7A) based on CIBERSORT revealed that the low-risk group had more infiltrating immune cells (e.g., CD8 T cells); nevertheless, resting natural killer cells, M0 and M1 macrophages, and activated mast cells were more enriched in the high-risk group. Comparing the differences in immune functions, the low-risk group appeared to have a more activated immune function, including checkpoint, cytolytic activity, HLA, inflammation promotion, T-cell coinhibition, and T-cell costimulation (Figure 7B). Therefore, considering these results of immune-related analysis, patients in the low-risk group may be more sensitive to immunotherapy.

3.6 | Assessment of chemotherapeutic effects

The IC50 value was used to predict the chemotherapeutic effects of cisplatin, docetaxel, gemcitabine, and paclitaxel. As indicated by the Spearman correlation analysis (Figure 8A–F), the risk score was negatively correlated with the IC50 values of docetaxel, gemcitabine, and paclitaxel, suggesting that patients in the high-risk group may exhibit a better chemotherapeutic response to these chemotherapy agents. However, for cisplatin, there were no significant differences in IC50 between the two risk groups (Figure 8G,H).

3.7 | PPI network and single gene analysis

A PPI network was established from the expression profiles of DEGs by the STRING online database (Figure 9A) and was visualized by Cytoscape software (Figure 9B). As presented in Figure 9A, genes associated with increased risk are shown in red markers, while the green markers represent genes that were enriched in the low-risk groups. Assessed by cytoHubba, the top 10 hub genes were obtained, ranking by degree method as LCE3D, SPRR3, SPRR2E, SPRR2G, LCE3E, FLG, SPRR2B, LCE2B, KPRP, and LCE6A. Survival analysis of the top five hub genes indicated that the expression of SPRR3 was notably associated with the prognosis of HNSCC patients (Figure 9C). Furthermore, the relationship between SPRR3 expression and immune cell infiltration was compared between the low- and high-expression groups (Figure 9D). The numbers of naïve B cells, plasma cells, regulatory T cells (Tregs), and neutrophils were markedly infiltrated in tumors with high SPRR3 expression; nevertheless, macrophage M2 was notably higher in tumors with low SPRR3 expression.

4 | DISCUSSION

HNSCC is a common malignant and aggressive cancer of the head and neck with a high incidence. Its atypical early clinical symptoms and insidious disease progression have increased morbidity and mortality over the years. Cancer cells and immune cells are prone to metabolic reprogramming under the regulation of various factors in the tumor microenvironment (TME), leading to abnormal activation of oncogenic signaling pathways. FAM is one of the key metabolic pathways in HNSCC reprogramming. However, studies demonstrating the relationship between FAMGs and HNSCC are still limited so far.

In this study, we established a risk score model for HNSCC prediction by analyzing the differential expression of FAMGs in both tumor and normal samples of the TCGA-HNSC cohort. By analyzing the ROC curve, it is evident that this model is superior to the other clinical factors for survival rate prediction of HNSCC patients. The nomogram analyzed by combining this model with clinical features is highly stable and accurate, contributing to higher predictive potential.

Furthermore, we screened 20 genes as prognostic markers following DEFAMGs analysis. HPSh1 has been reported to be an early detected gene that can be used as a biomarker for HNSCC identification. Similarly, the mRNA expression levels of LDHA isozymes were significantly greater in HNSCC compared to the controls. Martin et al. reported that ELOVL6 was involved in fatty acid elongation, and its low expression could improve the prognosis of HNSCC. Moreover, Thomas and colleagues developed a new risk
signature model with genes such as PRKAA2 to predict HNSCC prognosis.  

In fact, PRKAA2 is a related gene in the mTOR signaling pathway associated with chemotherapy resistance. In the literature, PRKAA2 has been shown to play a key role in the PKA-AMPK pathway, and its downregulation under certain drugs may prevent the occurrence of oral cancer. In this study, we found the levels of gene mutations in both high- and low-risk groups varied. Gene mutations may affect the risk score results. For instance, TP53 gene mutation will improve the risk score. The number of gene mutations present in the tumor is usually expressed by the TMB. Consequently, a combined analysis of TMB and risk score indicated that the prognosis of HNSCC patients with low TMB and low risk was relatively good, while high TMB and high risk significantly reduced the survival probability of the patients.

It has been reported that the interaction between the surrounding tissue matrix and immune cells in the TME may be responsible for the high recurrence and metastasis rates of HNSCC. Therefore, analyzing immune infiltration in patients with high- and low-risk scores is beneficial to judge the efficacy of immunotherapy on HNSCC. Our results showed that patients with low-risk score had more infiltration of immune cells, such as CD8 T cells and Treg cells, than patients with low-risk score. By inhibiting immune checkpoints such as PD-1, CD8 T cells enhance cellular immune activity and improve sensitivity to immunotherapy. Treg cells, as inhibitory immune cells, were found to be elevated, which also demonstrated that patients with low-risk scores were more suitable for immunotherapy. Additionally, we found that low-risk groups had a more active immune function, which further demonstrated that immunotherapy may benefit patients with low-risk scores. Moreover, we examined differences in responses to four common chemotherapy drugs in high-risk groups and low-risk groups. The results showed that docetaxel and gemcitabine exhibited higher IC50 values in the low-risk groups. IC50 is the drug concentration that induces apoptosis in 50% of tumor cells, which can reflect the degree of drug resistance of tumor cells. This result suggests that chemotherapeutic drug therapy may be more suitable for patients with high-risk scores.

According to our analysis, SPRR3 is an essential gene for HNSCC since it is associated with HNSCC prognosis. Previous studies on oral squamous cell carcinoma (OSCC) have shown that SPRR3 expression decreases with increasing OSCC malignancy. For patients with esophageal squamous cells, SPRR3 has also been reported to be downregulated. Additionally, our results demonstrated more immune cells infiltrating in the groups with higher SPRR3 expression, including CD8 T cells and Treg cells. Luo et al. found that SPRR3 may act as a radiosensitizer to influence tumor sensitivity to chemoradiotherapy. Overall, these results may provide potential guidance for the treatment of HNSCC.

Generally, this prognostic scoring model based on FAMGs can be used to predict the prognosis of HNSCC. Furthermore, this model can predict tumor sensitivity to chemotherapeutic drugs and feasibility of immunotherapy for HNSCC. Consequently, our study provides an accurate and reliable predictive model for HNSCC prognosis and sensitivity to chemotherapy and immunotherapy. These findings may offer personalized treatment strategies and increase the efficacy of clinical therapy for HNSCC, which encourages more personalized cancer therapies in the future.

However, this study still has some limitations. First, although the risk prediction model has a high success rate, a lack of clinical data can affect the predictive value of this model to some extent. Second, our study is bioinformatics-based and lacks biological trials to further support the conclusions. Nonetheless, the data analyzed in our study were from external cohorts, and thus the accuracy and reliability of our results were reliable.

5 CONCLUSION

In summary, a FAM prognostic risk score model was successfully established. This model can not only predict the survival of HNSCC patients but also assess the sensitivity and effectiveness of patients to immunotherapy and chemotherapy. This provides a certain guiding value for clinical practice and individualized treatment for HNSCC.

AUTHOR CONTRIBUTIONS

All persons designated as the authors have participated sufficiently in the work to take public responsibility for the content of the manuscript. All the authors ensure that they all gave substantial contributions.

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DATA AVAILABILITY STATEMENT

The data used to support the findings of this study comes from the public database and are available from the corresponding author upon request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.