Exploration of Medicine

Open Exploration

Open Access Original Article

Prognostic and diagnostic value of *SPINK* mRNAs expression in head and neck squamous cell carcinoma based on genome-wide analysis

Chao Ma[1*], Haoyu Li[2]

[1]Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China
[2]Department of Ophthalmology, the Second Xiangya Hospital of Central South University, Changsha 410011, Hunan, China

***Correspondence:** Chao Ma, Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University, 1 Jianshedong Rd, Zhengzhou 450052, Henan, China. gmchao219@163.com
Academic Editor: Apostolos Zaravinos, European University Cyprus, Cyprus
Received: June 14, 2024 **Accepted:** November 4, 2024 **Published:** December 2, 2024

Cite this article: Ma C, Li H. Prognostic and diagnostic value of *SPINK* mRNAs expression in head and neck squamous cell carcinoma based on genome-wide analysis. Explor Med. 2024;5:912–25. https://doi.org/10.37349/emed.2024.00265

Abstract

Aim: Head and neck squamous cell carcinoma (HNSC) is a major contributor to the global cancer burden. The serine protease inhibitor Kazal-type (*SPINK*) gene family has been linked to various cancers. This study explores the prognostic value of *SPINK* genes in predicting overall survival (OS) in HNSC patients.

Methods: We analyzed RNA sequencing and clinical data from 504 cancer and 44 non-cancer samples from the TCGA database. Differential expression and functional enrichment analyses gene ontology and Kyoto encyclopedia of genes and genomes (GO and KEGG) were performed using clusterProfiler. Protein-protein interaction (PPI) networks were built with STRING and visualized. Immune infiltration was evaluated using single-sample Gene Set Enrichment Analysis (ssGSEA). Survival analysis utilized Kaplan-Meier curves and Cox regression models.

Results: Our results showed that *SPINK5*, *SPINK7*, *SPINK8*, *SPINK9*, and *SPINK14* were significantly overexpressed in normal tissues compared to carcinoma tissues, whereas *SPINK1*, *SPINK4*, and *SPINK6* showed higher expression in carcinoma tissues. Correlation analysis revealed significant relationships among *SPINK* family members. GO and KEGG analyses highlighted their involvement in processes such as negative regulation of peptidase activity and serine-type endopeptidase inhibitor activity. PPI network analysis indicated close interactions between several SPINK proteins and other relevant proteins. Immune infiltration analysis showed that NK cells and Th2 cells were negatively correlated with *SPINK* genes, while mast cells and neutrophils were positively correlated. Survival analysis revealed that high mRNA expression levels of *SPINK1*, *SPINK5*, and *SPINK6* were significantly associated with OS in HNSC patients. Receiver operating characteristic (ROC) curve analysis indicated that these genes have diagnostic value. We developed a nomogram model that combines tumor stage and *SPINK* gene expression providing a predictive tool for patient prognosis.

Conclusions: This study elucidates the multifaceted roles of the *SPINK* gene family in HNSC. These findings offer valuable insights into their potential as diagnostic biomarkers and therapeutic targets.

Keywords

Biomarkers, diagnosis, genome-wide association study, head and neck squamous cell carcinoma

Introduction

Head and neck cancer is the sixth most common cancer and is a major source of the global cancer burden, accounting for about 6% of all cancer cases worldwide [1]. About 90% of head and neck cancers belong to the head and neck squamous cell carcinoma (HNSC) category [2]. There are more than 650,000 new HNSC cases annually worldwide, with 140,000 in Europe, and the incidence rate in men is higher than that in women [3, 4]. In 2018, there were reports in Europe that 3.1 percent of new HNSC patients were men, while the estimated mortality rate of HNSC in all cancer cases was 2.8 percent [5]. If HNSC is found at an early stage, there will be a higher cure rate in terms of 5-year survival rate. Over the past decade, knowledge about the molecular mechanisms driving tumor transformation and progression in HNSC has grown rapidly [6]. However, there are still some shortcomings, not only a limited number of biomarkers used in clinical practice, but also very few that have reached the stage of routine verification [7].

The serine protease inhibitor Kazal-type (*SPINK*) gene family consists of many family members. Presently, the members that have been discovered and identified include *SPINK1* to *SPINK12* [8]. The SPINK proteins are expressed in various tissues and help maintain the balance of protease activity by regulating serine proteases [9]. In addition, SPINK, a trypsin and trypsin-like inhibitor, contains at least 1 Kazal domain and 6 cysteines used to form 3 disulfide binding patterns [10, 11]. Studies have shown that the imbalance between SPINK protein and protease may lead to the occurrence of various cancers [12, 13]. However, in the context of HNSC, whether the *SPINK* family can be used as a prognostic biomarker has not been reported. Therefore, in this study, we will explore the prognostic value of mRNA expression in HNSC with a single *SPINK* family subunit.

Materials and methods

Data preparation

RNAseq data from the TCGA database (https://portal.gdc.cancer.gov) for the TCGA-HNSC project were downloaded and organized, including Trusted Platform Module (TPM) format data and clinical data (accessed by August 1, 2024). We conducted differential analysis on the original counts matrix using the DESeq2 package, following standard procedures [logFC (1) and *P*.adj (0.05)]. Additionally, we normalized the counts matrix using the variance stabilizing transformations method from the DESeq2 package. The HNSC expression data extracted from TCGA included 504 cancer mRNA sequences and 44 non-cancer mRNA sequences for subsequent analysis. The total number of samples with clinical information was 528. We visualize the results of the differential analysis using the ggplot2 package in R v4.2.1. The data for this study were obtained from the TCGA database and the use of this data did not require approval from the Ethics Committee. Data collection and application in accordance with TCGA release guidelines and data access policies.

SPINK family mRNA expression levels and correlation analyses

To clarify the expression differences of the *SPINK* gene family in HNSC, we performed a statistical analysis using the stats package in R v4.2.1. The results were visualized with the ggplot2 package. We assessed the co-expression relationship among *SPINK* genes using Pearson correlation coefficient analysis in R v4.2.1 and visualized the data with the ggplot2 package.

Functional analyses of *SPINK* genes

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analyses can yield gene expression data specifically for common *SPINK* genes. We utilized the clusterProfiler package in R v4.2.1 to conduct enrichment analysis, followed by data visualization with the ggplot2 package to clarify the

functions of the gene family. The functional analysis based on GO includes three categories: biological process (BP), cellular component (CC), and molecular functionality (MF).

Construction of protein-protein interaction and genetic interaction network

Protein-protein interaction (PPI) networks offer insights into the relationships among drugs, target genes, and proteins, as well as visual representations of this information. This study utilized the STRING tool and Cytoscape v3.10.2 to evaluate PPI, focusing on the functions and physical relationships of *SPINK* proteins. Genetic interaction (GI) networks leverage gene function prediction sites to elucidate complex interactions among relevant genes, generated through the Gene Multiple Association Network Integration Algorithm (GeneMANIA: https://genemania.org/, accessed by August 1, 2024). Both *SPINK* genes and the predicted genes were displayed concurrently for comparative analysis.

Immune infiltration analysis

Using the single-sample Gene Set Enrichment Analysis (ssGSEA) algorithm from the R package Gene Set Variation Analysis (GSVA), we calculated the immune infiltration status of the cloud dataset based on markers from 24 immune cells described in the immunity article [14]. Specific markers for the 24 immune cells can be found in the relevant references, and the violin plot illustrates the differences in immune cell abundance across different groups.

Survival analysis and diagnostic analysis

Patients were categorized into high-expression and low-expression groups based on the median value of each *SPINK* mRNA. The prognosis of HNSC was evaluated based on overall survival (OS). The log-rank test and Kaplan-Meier estimator were used to calculate the log-rank *P*-value and assess OS for the *SPINK* gene family. Clinical information, including gender and race, was also considered. We generated receiver operating characteristic (ROC) curves to evaluate the diagnostic value of the *SPINK* genes. For survival analysis, we utilized the survival package to conduct proportional hazards hypothesis testing and to fit a survival regression model. The results were visualized using the survivminer and ggplot2 packages. For the column charts, the survival package was used to test hypotheses regarding proportional hazards, and Cox regression analysis was performed. The rms package was utilized to create and visualize models related to nomograms. The survival curves, ROC curves, and nomograms were all generated using R v4.2.1.

Expression of *SPINK* gene family in tumor stages I–IV

We investigated the expression of the *SPINK* gene family across different tumor stages. To do this, we utilized the stats package in R v4.2.1 and applied the Kruskal-Wallis test and Dunn's test from the car package to analyze molecular expression differences among clinical variable groups.

Statistical analyses

R v4.2.1 was used to create correlation plots, survival curves, nomograms, ROC curves, and other data visualization. Furthermore, a P-value < 0.05 was deemed statistically significant.

Results

The mRNA expressions of *SPINK* in human normal tissues and carcinoma tissues

This study screened 10,666 differentially expressed genes (Figure 1). There are a total of 8 differentially expressed genes in the *SPINK* gene family, namely *SPINK1*, *SPINK4*, *SPINK5*, *SPINK6*, *SPINK7*, *SPINK8*, *SPINK9*, and *SPINK14* (Figure 2). In normal tissues, the mRNA expression levels of *SPINK5*, *SPINK7*, *SPINK8*, *SPINK9*, and *SPINK14* were significantly higher than in carcinoma tissues. Conversely, the mRNA expression levels of *SPINK1*, *SPINK4*, and *SPINK6* were higher in carcinoma tissues than in normal tissues.

Pearson correlation coefficient analysis was used to identify correlations in the mRNA expression levels of *SPINK* genes (Figure 3, Table S1). Asterisks denote correlated molecules, with red indicating positive correlations and blue indicating negative correlations. The strength of the correlations is

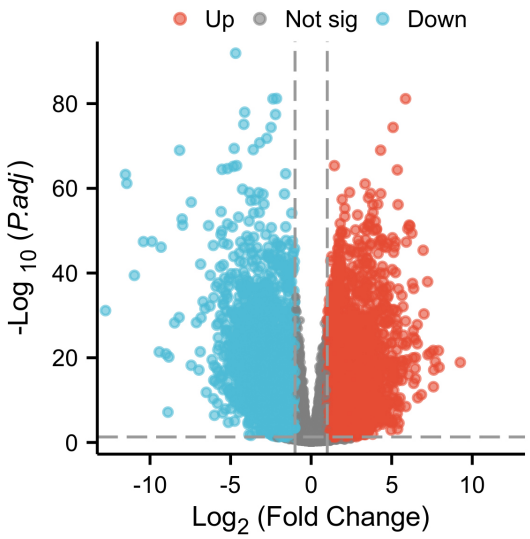

Figure 1. Differential expression analysis of the TCGA data set. Volcano plot of differential expression analysis of the HNSC data set. Not sig: genes that did not meet the significance criteria in the statistical analysis; HNSC: head and neck squamous cell carcinoma

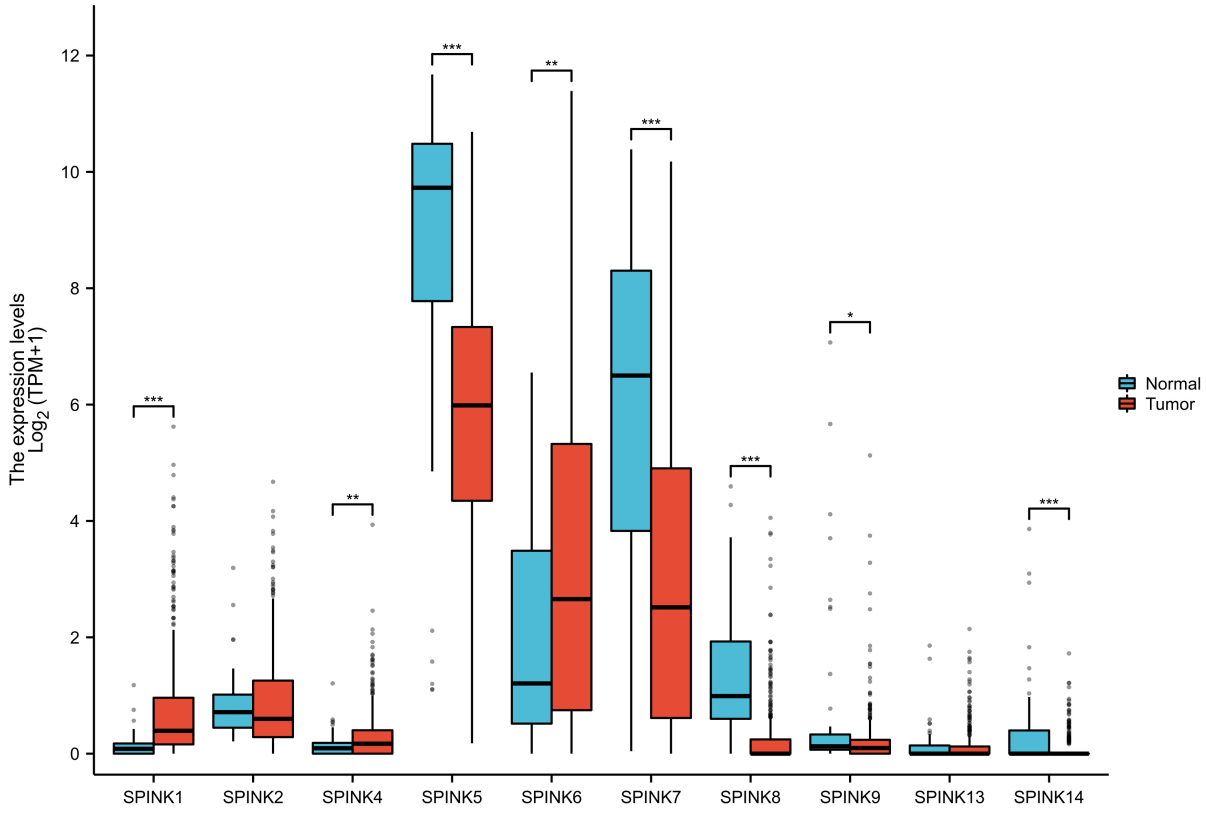

Figure 2. The boxplots about *SPINK* gene expressions in normal tissues and carcinoma tissues. Boxplot for *SPINK1*, *SPINK4*, *SPINK5*, *SPINK6*, *SPINK7*, *SPINK8*, *SPINK9*, and *SPINK14* expressions. *SPINK*: serine protease inhibitor Kazal-type; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

categorized as follows: $|r| > 0.95$ indicates significant correlation; $|r| \geq 0.8$ indicates high correlation; $0.5 \leq |r| < 0.8$ indicates moderate correlation; $0.3 \leq |r| < 0.5$ indicates low correlation; $|r| < 0.3$ indicates weak correlation. Genes in the *SPINK* gene family that show a correlation greater than 0.5 include *SPINK5* with *SPINK7* and *SPINK6* with *SPINK7*.

SPINK genes' function, pathway, and co-expression enrichment analyses

The biological functions of *SPINK* genes were identified using Database for Annotation, Visualization, and Integrated Discovery (DAVID) based on GO and KEGG analyses (Figure 4A, Table S2). The analysis revealed that *SPINK* genes were primarily enriched in several categories: They are involved in the negative

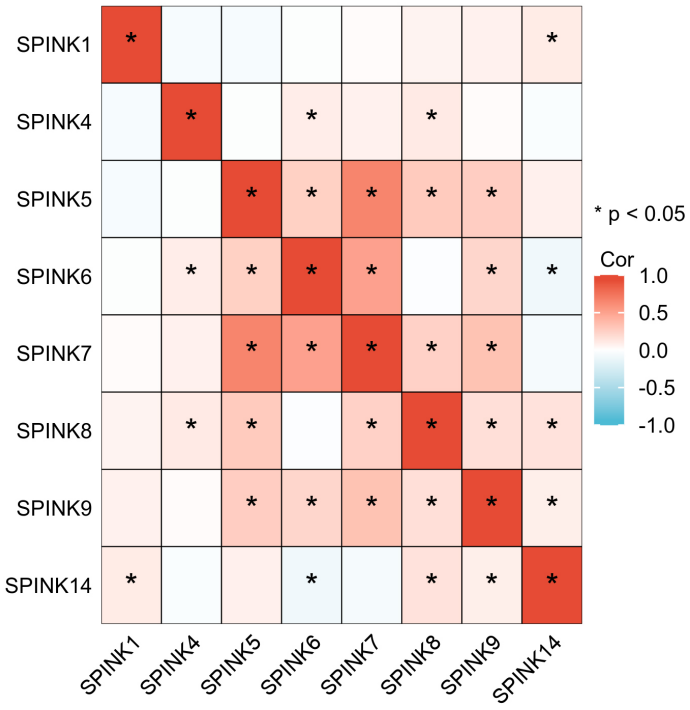

Figure 3. Pearson correlation coefficients for *SPINK* gene expression levels. The darker the color the greater the correlation. *SPINK*: serine protease inhibitor Kazal-type

regulation of peptidase activity, endopeptidase activity, proteolysis, and hydrolase activity of BP; lamellar body of CC; serine-type endopeptidase inhibitor activity, peptidase inhibitor activity, endopeptidase inhibitor activity, and endopeptidase regulator activity of MF. However, no relevant pathways were identified as enriched in the KEGG analysis.

The protein level gene co-expression is illustrated using a PPI network via STRING (Figure 4B). *SPINK7*, *6*, *4*, and *14* exhibit stronger associations with other proteins. A GI network illustrating the interactions among the mRNA expressions of *SPINK*s is presented through GeneMANIA (Figure 4C). The top 10 most related genes include *SPINK13*, *RECK*, *SPINK2*, *FST*, *FSTL3*, *FSTL4*, *SLCO1B3-SLCO1B7*, *MSANTD3-TMEFF1*, *SLCO4C1*, and *SLCO5A1*.

Immune infiltration analysis

To further investigate the role of immune cells in HNSC, we conducted a separate analysis of the TCGA dataset. The analysis of immune cell correlations revealed that the HNSC and *SPINK* gene families had a significant negative correlation with NK cells and Th2 cells. In contrast, mast cells showed a positive correlation with neutrophils (see Figure 5 and 6).

Survival analysis, diagnostic analysis, and *SPINK* gene family in tumor stages I–IV

The prognostic significance of *SPINK* mRNA expressions was assessed using R v4.2.1. *SPINK1*, *5*, and *6* mRNA expressions were significantly associated with OS in HNSC patients (P = 0.005, 0.006, and 0.003, respectively; Figure 7). In addition to these three genes, the mRNA expressions of the remaining *SPINK* genes did not show significant (P > 0.05) correlations with the OS in HNSC patients (Figure 7). The ROC curves showed *SPINK1*, *5*, *7*, and *8* mRNA expressions were closely related to the occurrence of HNSC (AUC = 0.793; AUC = 0.817; AUC = 0.728; AUC = 0.859; Figure 8). Notably, both *SPINK1* and *5* served as simultaneous diagnostic and prognostic markers. A nomogram was created to predict the survival probability of patients with HNSC (Figure 9). The C-index for this model was 0.669. Although it did not meet the 0.7 threshold, we believe that the combined analysis of the *SPINK* gene family can contribute to the prognosis prediction for HMSC patients. The model incorporated the tumor stage, the *SPINK* gene family and the annual survival rate. In this study, stage I and II were classified as 'early stage', and stages III and IV were classified as 'late stage'. The results showed that *SPINK1*, *4*, *5*, and *9* were associated with the tumor staging of HNSC (Figure 10).

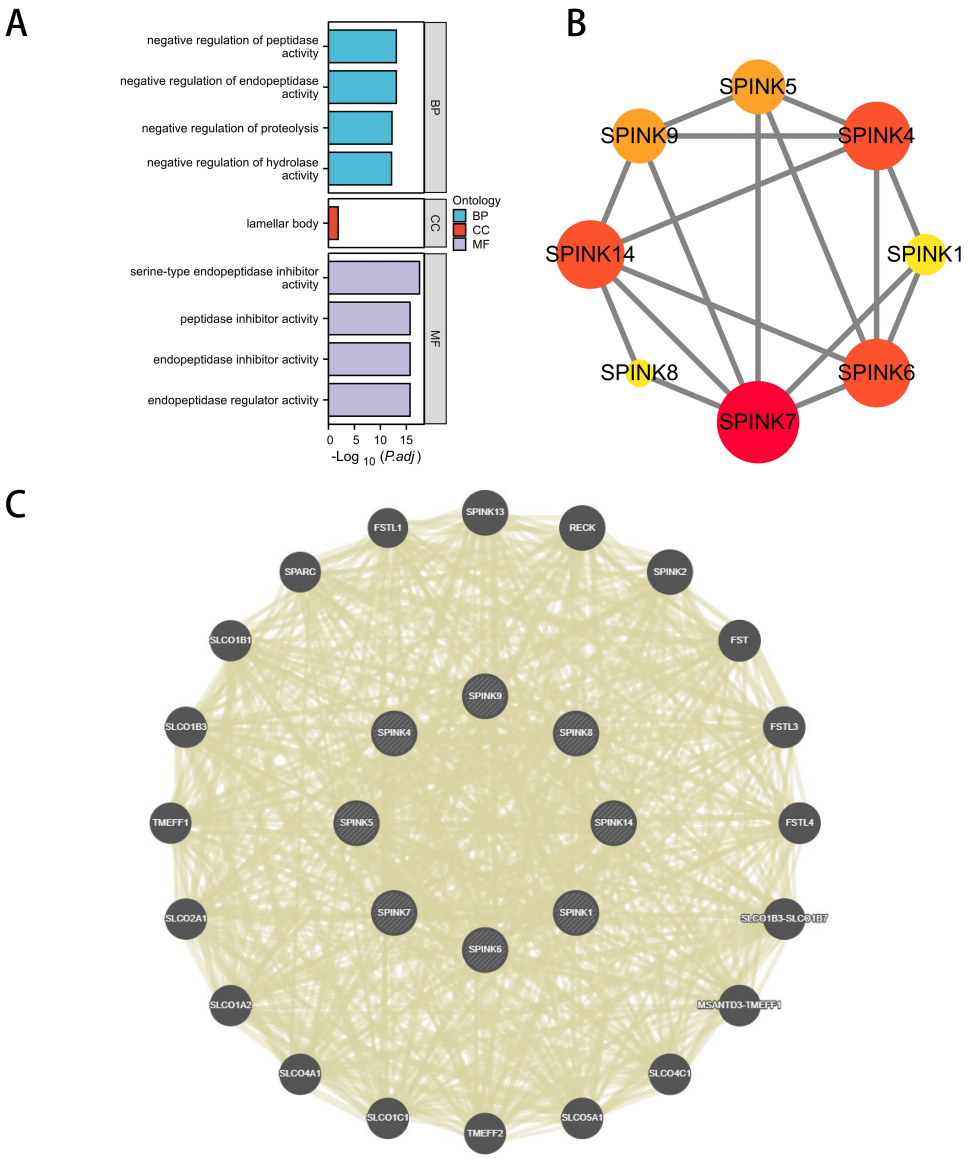

Figure 4. Functional analysis. (**A**) GO analysis for *SPINK* genes. (**B**) PPI network. The darker the color and the larger the graphic, the closer the connection with other proteins. (**C**) GGI network. GO: gene ontology; *SPINK*: serine protease inhibitor Kazal-type; PPI: protein-protein interaction; GGI: genetic-genetic interaction; BP: biological process; CC: cellular component; MF: molecular functionality

Discussion

This study evaluated the prognostic significance of the *SPINK* gene family using the TCGA database for HNSC. High expression levels of *SPINK1,* along with low levels of *SPINK5*, *SPINK7*, and *SPINK8* were associated with improved OS in patients with HNSC. The mRNA expression levels of *SPINK1*, *4*, *5*, *6*, *7*, *8*, *9*, and *14* can help predict the development of HNSC. In addition, we performed GO functional analysis to explore the relationships between genes and proteins, aiming to predict the functions of the *SPINK* gene family.

*SPINK*s are a family of protein molecules. Protease inhibitors are crucial for regulating protein hydrolysis activity. Besides acting as antiproteases, they also possess significant anti-inflammatory, pro-inflammatory properties, and antibacterial properties [15]. The *SPINK* gene family is crucial in the development of various diseases, including certain types of cancer. Mature *SPINK1* consists of 56 amino acids, also known as islet trypsin inhibitor (*PSTI*) or tumor-associated trypsin inhibitor (*TATI*). Recent studies indicate that *SPINK1* promotes tumor cell growth in various cancers, including lung adenocarcinoma [16], colon [17, 18], pancreas [19], and ovarian cancer [20]. This indicates that *SPINK1* may promote cancer through a mechanism different from its classical activity as a serine protease inhibitor.

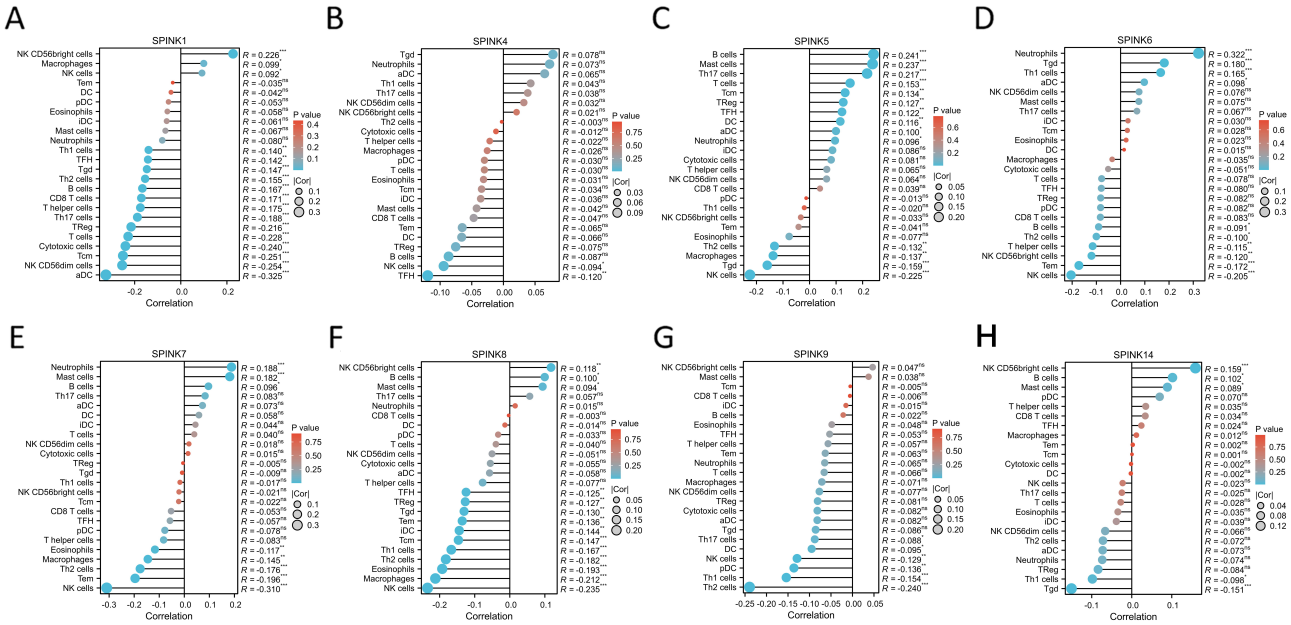

Figure 5. 24 immune cells infiltrated. The X coordinate represents the correlation coefficient, and the Y coordinate represents the names of 24 immune cells. *SPINK1* (**A**), *SPINK4* (**B**), *SPINK5* (**C**), *SPINK6* (**D**), *SPINK7* (**E**), *SPINK8* (**F**), *SPINK9* (**G**) and *SPINK14* (**H**). The left side is negatively correlated, and the right side is positively correlated, the larger the spherical shape, the higher the correlation coefficient, and the darker the color, the greater the *P*-value. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant

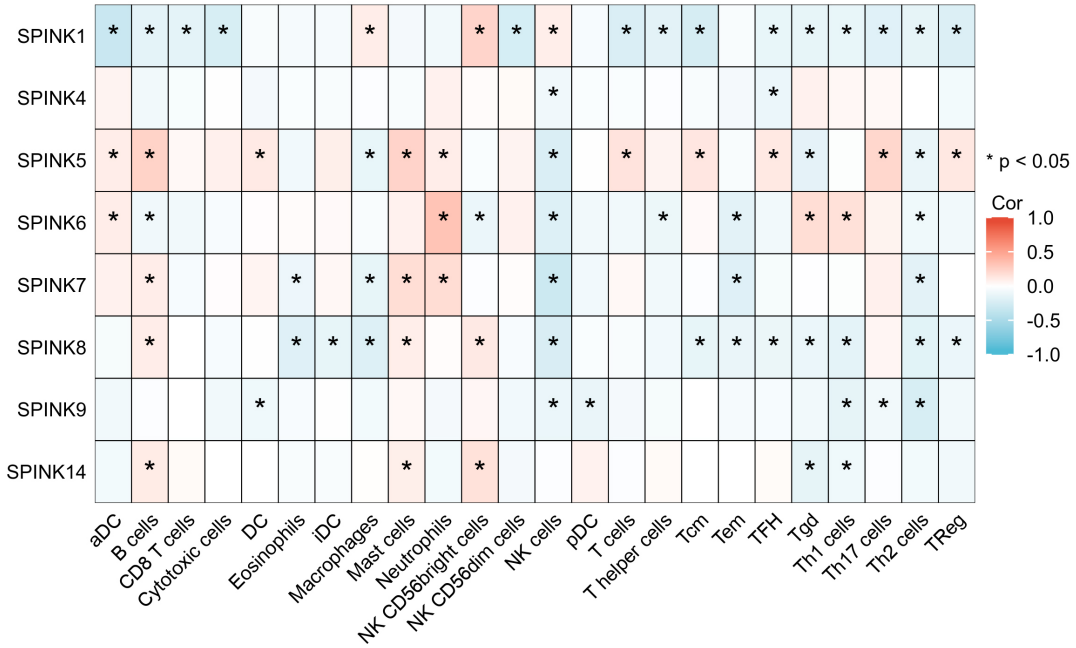

Figure 6. 24 immune cells infiltrated. The relationship between the *SPINK* gene family is positively correlated in red and negatively correlated in blue. The darker the color, the stronger the correlation coefficient and the * represents statistical significance

SPINK2 is mainly synthesized in testes and seminal vesicles. It plays a role in the reproductive process. The expression of *SPINK2* was significantly increased in primary cutaneous large B-cell lymphoma, particularly in the germinal center of B-cell-like lymphoma and activated B-cell-like diffuse large B-cell lymphoma. This suggests a close association between *SPINK2* and the pathogenesis of primary cutaneous large B-cell lymphoma [12]. There also is a study indicating the downregulation of *SPINK2* in HNSC [21]. *SPINK4* was originally isolated from pig intestines and was later found to be highly expressed in goblet cells in the recess of Lieberkühn in humans and pigs, as well as in monocytes and the central nervous system [22–24]. *SPINK5* expression has been documented in the thymic and vaginal epithelium, Bartolin's gland, oral mucosa, tonsil, and parathyroid gland [25]. The abnormal expression of *SPINK5* is also related to the

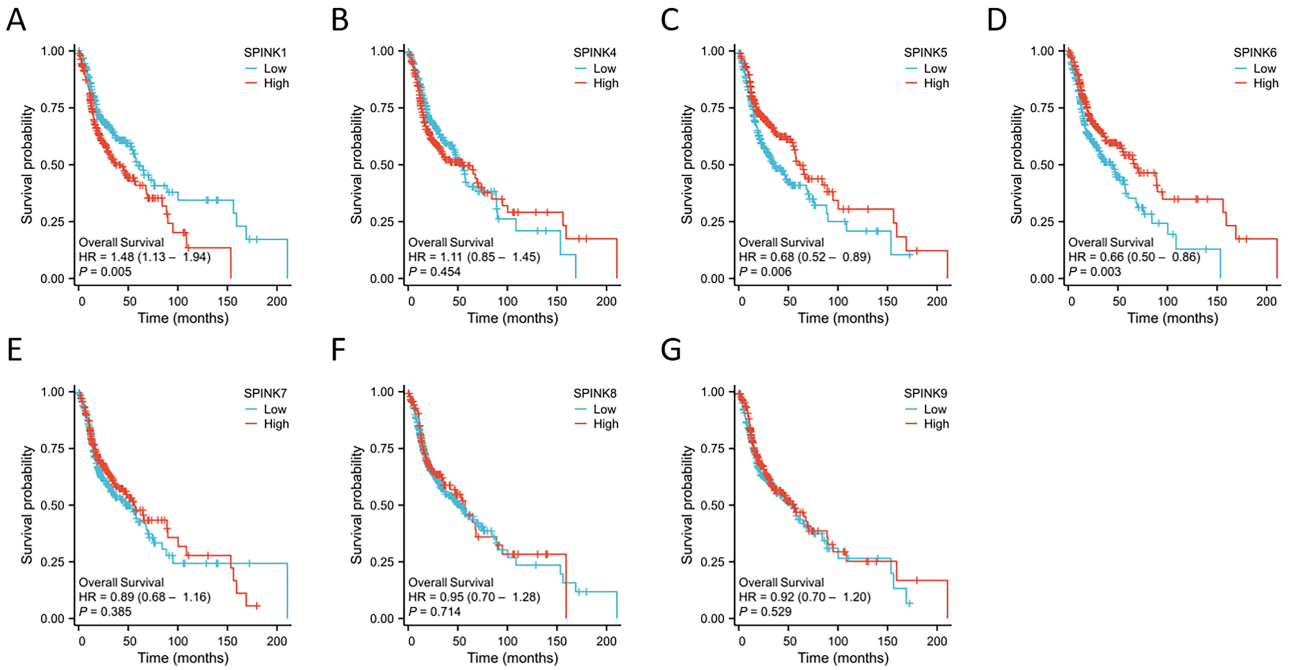

Figure 7. The Kaplan-Meier survival curves of *SPINK* genes for OS of HNSC patients. Survival curves for 528 HNSC patients according to *SPINK1* (**A**), *SPINK4* (**B**), *SPINK5* (**C**), *SPINK6* (**D**), *SPINK7* (**E**), *SPINK8* (**F**) and *SPINK9* (**G**) expressions. *SPINK*: serine protease inhibitor Kazal-type; OS: overall survival; HNSC: head and neck squamous cell carcinoma

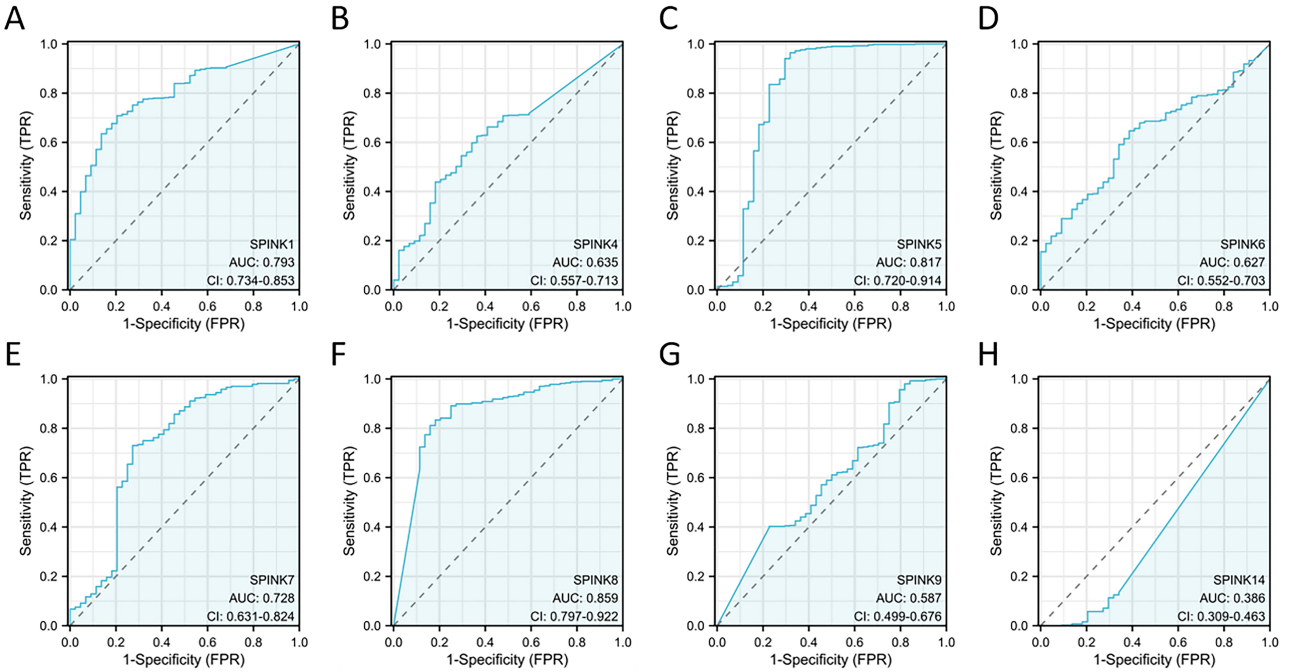

Figure 8. ROC curves for *SPINK* genes in HNSC. ROC curves for 528 HNSC patients according to *SPINK1* (**A**), *SPINK4* (**B**), *SPINK5* (**C**), *SPINK6* (**D**), *SPINK7* (**E**), *SPINK8* (**F**), *SPINK9* (**G**) and *SPINK14* (**H**) expressions. ROC: receiver operating characteristic; *SPINK*: serine protease inhibitor Kazal-type; HNSC: head and neck squamous cell carcinoma

pathogenesis of certain tumors [26]. Studies indicate that in patients with oral squamous cell carcinoma and esophageal squamous cell carcinoma who have elevated *SPINK5* expression have shorter survival times compared to those with low expression. *SPINK5* may serve as an independent prognostic factor for tumor development [27, 28]. *SPINK6* has a broad spectrum, not only in the skin to control kallikrein activity, but also in other tissues to control kallikrein activity [29]. Additionally, *SPINK6* is a known prognostic factor for HNSC [30]. The *SPINK7* gene, a tumor suppressor gene, regulates proteasome cascade during carcinogenesis and invasion of esophageal cancer through the urokinase-type plasmin activator/plasmin MAP kinase signaling pathway [31]. A recent study has shown that a large number of *SPINK9* positive staining was observed in squamous cell carcinoma [32]. These results indicate that *SPINK9* expression may

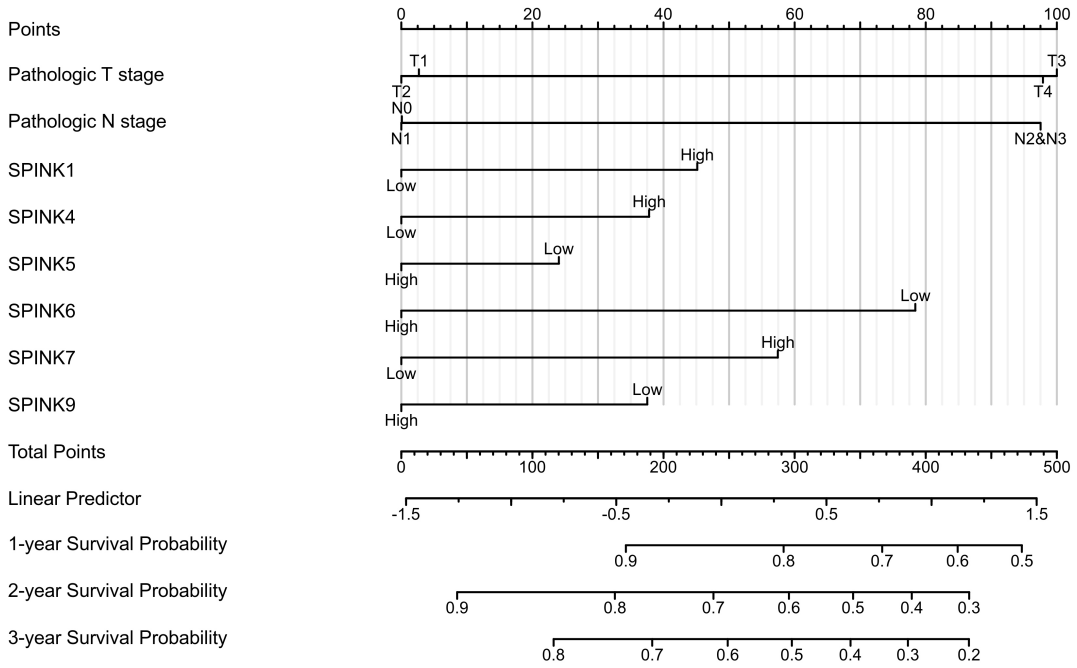

Figure 9. Nomogram for the relationship between medical data and risk score in HNSC patients. *SPINK*: serine protease inhibitor Kazal-type; HNSC: head and neck squamous cell carcinoma

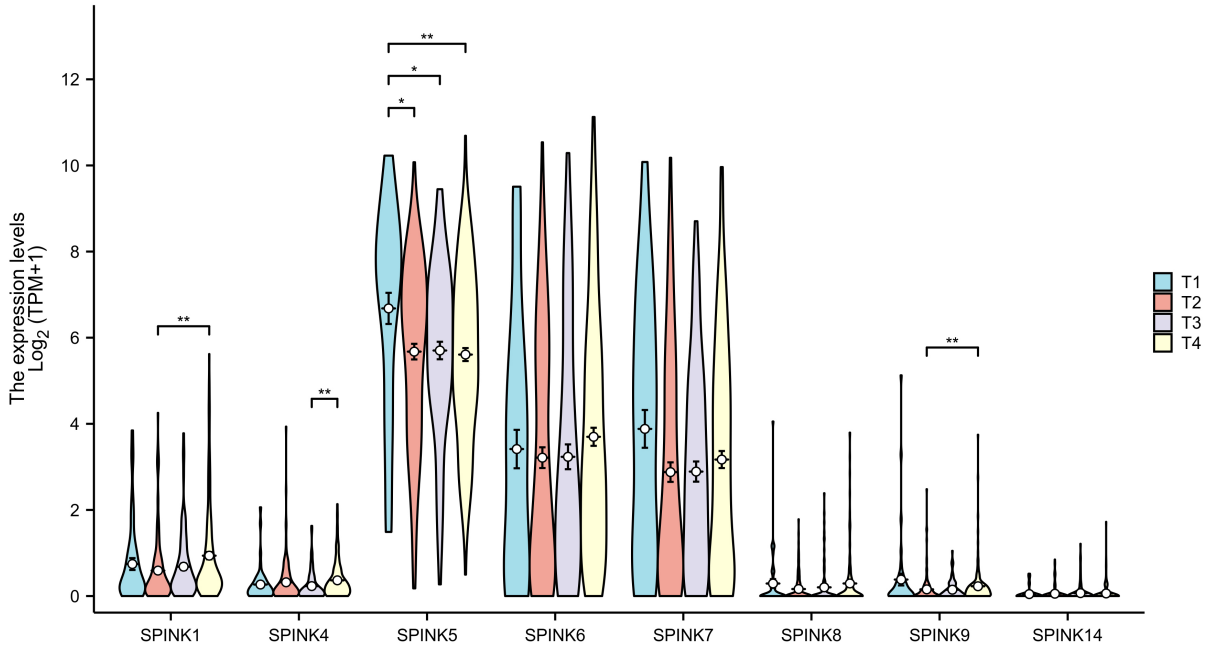

Figure 10. The expression of the *SPINK* gene family in different tumor stages. The four colors represent the T1–T4 stages of the tumor, and the groups with differences are marked with asterisks in the figure. *SPINK*: serine protease inhibitor Kazal-type; *: $P < 0.05$; **: $P < 0.01$

be related to the progression of squamous cell carcinoma.

The analysis of the *SPINK* gene family in HNSC has provided significant insights into their roles in different BPs. Notably, the GO and KEGG enrichment analyses have shown that *SPINK* genes are predominantly involved in the negative regulation of peptidase activity, proteolysis, and hydrolase activity. These findings are consistent with existing literature that highlights the critical roles of peptidase inhibitors in cancer progression and immune regulation. For instance, the study by Huang et al. [33] demonstrates that the parathyroid hormone-like hormone (PTHLH)-activated network in hepatocellular carcinoma negatively regulates peptidase activity. The regulation is vital for inducing apoptosis and protein catabolism. This suggests that similar regulatory mechanisms might exist in HNSC, where *SPINK* genes may influence apoptosis and proteolysis by inhibiting peptidases activity.

Moreover, the regulation of peptidase activity in immune responses is well-established. For example, the study by Tanaka et al. [34] demonstrates that soluble CD26 enhances T-cell proliferation through its peptidase activity. This suggests that regulating peptidase activity significantly affects immune cell function. In the context of HNSC, our findings that *SPINK* genes are involved in immune cell infiltration further support the idea that these genes may influence tumor immunity by modulating peptidase activity.

The STRING analysis has identified key interactions between *SPINK* proteins and other regulatory molecules, such as BRCA1 and DKK1, both of which are involved in cancer-related pathways [35]. This complex network of interactions highlights the diverse roles of *SPINK* genes in cellular processes. These include cell cycle regulation, apoptosis, and immune responses. Furthermore, the role of *SPINK* genes in proteolytic processes is supported by research on α2-macroglobulins, which are broad-spectrum endopeptidase inhibitors involved in various biological functions, such as defending against external toxins and regulation of cytokines and growth factors [36]. This supports the idea that *SPINK* genes may act as critical modulators in the tumor microenvironment by regulating protease activity and influencing signaling pathways.

Our analysis of immune infiltration in HNSC revealed significant associations between *SPINK* gene family expression and different immune cell types, especially NK cells and Th2 cells. NK cells are essential components of the innate immune system and are known for targeting and destroying malignant cells without prior sensitization. Recent research has highlighted their role in tumor immunosurveillance and potential therapeutic applications in HNSC. For instance, a study created a prognostic model based on NK cell-related genes, demonstrating that NK cell infiltration is vital for positive patient outcomes and responsiveness to immunotherapy in HNSC [37]. Additionally, another study identified germline variants in NK cells that influence tumor immune microenvironment subtypes and patient prognosis, highlighting their significance in cancer immunity [38]. Our findings indicate that NK cells are significantly negatively correlated with *SPINK* gene family expression in HNSC. This inverse relationship suggests that increased *SPINK* gene expression could lead to an immunosuppressive tumor microenvironment. This may reduce NK cell activity and promote tumor progression. This finding is consistent with observations that the immune landscape of the tumor microenvironment, including NK cell recruitment, is critical for HNSC prognosis and therapeutic response [39]. Furthermore, the identified immune-related genes and pathways, such as NK cell-mediated cytotoxicity, are important factors in lipid metabolism-mediated tumor immunity in HNSC. This underscores the complex relationship between metabolic processes and immune regulation [40].

Th2 cells, a component of the adaptive immune system, promote humoral immunity and are often associated with anti-inflammatory responses. Our analysis showed a significant negative correlation between Th2 cells and *SPINK* gene expression, suggesting that higher *SPINK* levels may inhibit Th2 cell-mediated immune responses. This is consistent with findings from Tan et al. [41], who reported that specific transcription factors in HNSC are associated with immune cell infiltration, including NK and Th2 cells, and influence tumor progression and patient outcomes.

This study found that higher mRNA expression levels of *SPINK1*, *SPINK5*, and *SPINK6* were related to favorable OS. We constructed a nomogram that indicated the *SPINK* gene family can participate in predicting scores. The contribution of tumor staging increased with the progress of the tumor, and the younger the patients tend to have higher scores. Compared with these risk-related factors, the importance of tumor staging significantly outweighs that of other effects. Using this model, we can predict the time-dependent survival rate. Patients with lower total scores had better 1-, 3-, and 5-year survival rates compared to those with higher total scores. Previous research has identified a few biomarkers for HNSC detection, including programmed cell death-ligand 1 and vascular endothelial growth factor [6]. In this study, the ROC curve analysis showed that the expression levels of *SPINK1*, *5*, *7*, and *8* can distinguish between adjacent normal tissues and HNSC tissues. Moreover, *SPINK1*, *4*, *5*, and *9* were linked to the tumor staging of HNSC. These findings may provide valuable insights for diagnosing tumor metastasis in clinical practice.

This study systematically explored the multifaceted roles of the *SPINK* gene family in head and HNSC. It examined expression levels, functional roles, PPI, immune infiltration, survival prognosis, and differential expression across tumor stages. The findings provide valuable insights into the potential mechanisms of the *SPINK* gene family in HNSC and highlight their significance in disease progression and prognosis. Future research, incorporating wet-lab validation and clinical studies, is essential to confirm these findings and clearly define the therapeutic and diagnostic potential of the *SPINK* gene family in HNSC.

Abbreviations

BP: biological process

CC: cellular component

GI: genetic interaction

GO: gene ontology

HNSC: head and neck squamous cell carcinoma

KEGG: Kyoto encyclopedia of genes and genomes

MF: molecular functionality

OS: overall survival

PPI: protein-protein interaction

ROC: receiver operating characteristic

SPINK: serine protease inhibitor Kazal-type

Supplementary materials

The supplementary Table S1 for this article is available at: https://www.explorationpub.com/uploads/Article/file/1001265_sup_1.xlsx. The supplementary Table S2 for this article is available at: https://www.explorationpub.com/uploads/Article/file/1001265_sup_2.pdf.

Declarations

Author contributions

CM: Conceptualization, Writing—original draft, Writing—review & editing, Validation, Supervision. HL: Conceptualization, Investigation, Writing—original draft.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The data was obtained from the TCGA public database and has no privacy or ethical implications. Ethics approval is therefore not required.

Consent to participate

The data was obtained from the TCGA public database and has no privacy or ethical implications. Consent to participate is therefore not required.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Funding

Not applicable.

References

1. Riva G, Pecorari G, Biolatti M, Pautasso S, Lo Cigno I, Garzaro M, et al. PYHIN genes as potential biomarkers for prognosis of human papillomavirus-positive or -negative head and neck squamous cell carcinomas. Mol Biol Rep. 2019;46:3333–47. [DOI] [PubMed]
2. Rahman S, Kraljević Pavelić S, Markova-Car E. Circadian (De)regulation in Head and Neck Squamous Cell Carcinoma. Int J Mol Sci. 2019;20:2662. [DOI] [PubMed] [PMC]
3. Gatta G, Botta L, Sánchez MJ, Anderson LA, Pierannunzio D, Licitra L, et al.; EUROCARE Working Group:. Prognoses and improvement for head and neck cancers diagnosed in Europe in early 2000s: The EUROCARE-5 population-based study. Eur J Cancer. 2015;51:2130–43. [DOI] [PubMed]
4. Duran G, Aguín S, Cruz R, Barros F, Giráldez JM, Bernárdez B, et al. Association of GSTP1 and ERCC1 polymorphisms with toxicity in locally advanced head and neck cancer platinum-based chemoradiotherapy treatment. Head Neck. 2019;41:2704–15. [DOI] [PubMed]
5. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer. 2018;103:356–87. [DOI] [PubMed]
6. Lampri ES, Chondrogiannis G, Ioachim E, Varouktsi A, Mitselou A, Galani A, et al. Biomarkers of head and neck cancer, tools or a gordian knot? Int J Clin Exp Med. 2015;8:10340–57. [PubMed] [PMC]
7. Budach V, Tinhofer I. Novel prognostic clinical factors and biomarkers for outcome prediction in head and neck cancer: a systematic review. Lancet Oncol. 2019;20:e313–26. [DOI] [PubMed]
8. Ohmuraya M, Yamamura K. The Roles of Serine Protease Inhibitor Kazal Type 1 (*SPINK*1) in Pancreatic Diseases. Exp Anim. 2011;60:433–44. [DOI] [PubMed]
9. Lu SM, Lu W, Qasim MA, Anderson S, Apostol I, Ardelt W, et al. Predicting the reactivity of proteins from their sequence alone: Kazal family of protein inhibitors of serine proteinases. Proc Natl Acad Sci U S A. 2001;98:1410–5. [DOI] [PubMed] [PMC]
10. Rawlings ND, Tolle DP, Barrett AJ. Evolutionary families of peptidase inhibitors. Biochem J. 2004;378:705–16. [DOI] [PubMed] [PMC]
11. Laskowski M Jr, Kato I. Protein inhibitors of proteinases. Annu Rev Biochem. 1980;49:593–626. [DOI] [PubMed]
12. Hoefnagel JJ, Dijkman R, Basso K, Jansen PM, Hallermann C, Willemze R, et al. Distinct types of primary cutaneous large B-cell lymphoma identified by gene expression profiling. Blood. 2005;105:3671–8. [DOI] [PubMed]
13. Stenman UH. Role of the tumor-associated trypsin inhibitor *SPINK*1 in cancer development. Asian J Androl. 2011;13:628–9. [DOI] [PubMed] [PMC]
14. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics. 2013;14:7. [DOI] [PubMed] [PMC]
15. Laflamme BA, Wolfner MF. Identification and function of proteolysis regulators in seminal fluid. Mol Reprod Dev. 2013;80:80–101. [DOI] [PubMed] [PMC]
16. Xu L, Lu C, Huang Y, Zhou J, Wang X, Liu C, et al. *SPINK*1 promotes cell growth and metastasis of lung adenocarcinoma and acts as a novel prognostic biomarker. BMB Rep. 2018;51:648–53. [DOI] [PubMed] [PMC]
17. Ida S, Ozaki N, Araki K, Hirashima K, Zaitsu Y, Taki K, et al. *SPINK*1 Status in Colorectal Cancer, Impact on Proliferation, and Role in Colitis-Associated Cancer. Mol Cancer Res. 2015;13:1130–8. [DOI] [PubMed]

18. Tiwari R, Pandey SK, Goel S, Bhatia V, Shukla S, Jing X, et al. *SPINK*1 promotes colorectal cancer progression by downregulating Metallothioneins expression. Oncogenesis. 2015;4:e162. [DOI] [PubMed] [PMC]
19. Zhang J, Wang D, Hu N, Wang Q, Yu S, Wang J. The construction and proliferative effects of a lentiviral vector capable of stably overexpressing *SPINK*1 gene in human pancreatic cancer AsPC-1 cell line. Tumour Biol. 2016;37:5847–55. [DOI] [PubMed]
20. Mehner C, Oberg AL, Kalli KR, Nassar A, Hockla A, Pendlebury D, et al. Serine protease inhibitor Kazal type 1 (*SPINK*1) drives proliferation and anoikis resistance in a subset of ovarian cancers. Oncotarget. 2015;6:35737–54. [DOI] [PubMed] [PMC]
21. Du P, Chai Y, Zong S, Yue J, Xiao H. Identification of a Prognostic Model Based on Fatty Acid Metabolism-Related Genes of Head and Neck Squamous Cell Carcinoma. Front Genet. 2022;13: 888764. [DOI] [PubMed] [PMC]
22. Agerberth B, Söderling-Barros J, Jörnvall H, Chen ZW, Ostenson CG, Efendić S, et al. Isolation and characterization of a 60-residue intestinal peptide structurally related to the pancreatic secretory type of trypsin inhibitor: influence on insulin secretion. Proc Natl Acad Sci U S A. 1989;86:8590–4. [DOI] [PubMed] [PMC]
23. Metsis M, Cintra A, Solfrini V, Ernfors P, Bortolotti F, Morrasutti DG, et al. Molecular cloning of PEC-60 and expression of its mRNA and peptide in the gastrointestinal tract and immune system. J Biol Chem. 1992;267:19829–32. [PubMed]
24. Norberg A, Gruber S, Angelucci F, Renlund S, Wadensten H, Efendic S, et al. Identification of the bioactive peptide PEC-60 in brain. Cell Mol Life Sci. 2003;60:378–81. [DOI] [PubMed] [PMC]
25. Mägert HJ, Ständker L, Kreutzmann P, Zucht HD, Reinecke M, Sommerhoff CP, et al. LEKTI, a Novel 15-Domain Type of Human Serine Proteinase Inhibitor. J Biol Chem. 1999;274:21499–502. [DOI] [PubMed]
26. Sun S, Su G, Zheng X. Inhibition of the Tumor Suppressor Gene *SPINK*5 via EHMT2 Induces the Oral Squamous Cell Carcinoma Development. Mol Biotechnol. 2024;66:208–21. [DOI] [PubMed]
27. Zhao C, Zou H, Zhang J, Wang J, Liu H. An integrated methylation and gene expression microarray analysis reveals significant prognostic biomarkers in oral squamous cell carcinoma. Oncol Rep. 2018; 40:2637–47. [DOI] [PubMed] [PMC]
28. Li Y, Lu Z, Che Y, Wang J, Sun S, Huang J, et al. Immune signature profiling identified predictive and prognostic factors for esophageal squamous cell carcinoma. Oncoimmunology. 2017;6:e1356147. [DOI] [PubMed] [PMC]
29. Plaza K, Kalinska M, Bochenska O, Meyer-Hoffert U, Wu Z, Fischer J, et al. Gingipains of Porphyromonas gingivalis Affect the Stability and Function of Serine Protease Inhibitor of Kazal-type 6 (*SPINK*6), a Tissue Inhibitor of Human Kallikreins. J Biol Chem. 2016;291:18753–64. [DOI] [PubMed] [PMC]
30. Li Z, Zheng C, Liu H, Lv J, Wang Y, Zhang K, et al. A novel oxidative stress-related gene signature as an indicator of prognosis and immunotherapy responses in HNSCC. Aging (Albany NY). 2023;15: 14957–84. [DOI] [PubMed] [PMC]
31. Cheng X, Shen Z, Yin L, Lu S, Cui Y. $ECRG_2$ Regulates Cell Migration/Invasion through Urokinase-type Plasmin Activator Receptor (uPAR)/ β1 Integrin Pathway. J Biol Chem. 2009;284:30897–906. [DOI] [PubMed] [PMC]
32. Redelfs L, Fischer J, Weber C, Wu Z, Meyer-Hoffert U. The serine protease inhibitor of Kazal-type 9 (*SPINK*9) is expressed in lichen simplex chronicus, actinic keratosis and squamous cell carcinoma. Arch Dermatol Res. 2016;308:133–7. [DOI] [PubMed]
33. Huang J, Wang L, Jiang M, Lin H, Qi L, Diao H. *PTHLH* coupling upstream negative regulation of fatty acid biosynthesis and Wnt receptor signal to downstream peptidase activity-induced apoptosis network in human hepatocellular carcinoma by systems-theoretical analysis. J Recept Signal Transduct Res. 2012;32:250–6. [DOI] [PubMed]

34. Tanaka T, Duke-Cohan JS, Kameoka J, Yaron A, Lee I, Schlossman SF, et al. Enhancement of antigen-induced T-cell proliferation by soluble CD26/dipeptidyl peptidase IV. Proc Natl Acad Sci U S A. 1994; 91:3082–6. [DOI] [PubMed] [PMC]
35. Goulas T, Garcia-Ferrer I, Marrero A, Marino-Puertas L, Duquerroy S, Gomis-Rüth FX. Structural and functional insight into pan-endopeptidase inhibition by α_2-macroglobulins. Biol Chem. 2017;398: 975–94. [DOI] [PubMed]
36. Zheng W, Xu HE, Johnston SA. The Cysteine-Peptidase Bleomycin Hydrolase Is A Member of the Galactose Regulon in Yeast. J Biol Chem. 1997;272:30350–5. [DOI] [PubMed]
37. Guo Z, Zhao Y, Xu M, Zhao L, Wang X. Natural killer cell-based signature: Prognostic analysis in head and neck squamous cell carcinoma. J Gene Med. 2024;26:e3671. [DOI] [PubMed]
38. Xu X, Li J, Zou J, Feng X, Zhang C, Zheng R, et al. Association of Germline Variants in Natural Killer Cells With Tumor Immune Microenvironment Subtypes, Tumor-Infiltrating Lymphocytes, Immunotherapy Response, Clinical Outcomes, and Cancer Risk. JAMA Netw Open. 2019;2:e199292. [DOI] [PubMed] [PMC]
39. Shu T, Wang X. Cuproptosis combines immune landscape providing prognostic biomarker in head and neck squamous carcinoma. Heliyon. 2023;9:e15494. [DOI] [PubMed] [PMC]
40. Liu S, Wang S, Wang Z. Identification of genetic mechanisms underlying lipid metabolism-mediated tumor immunity in head and neck squamous cell carcinoma. BMC Med Genomics. 2023;16:110. [DOI] [PubMed] [PMC]
41. Tan M, Lin X, Chen H, Ye W, Yi J, Li C, et al. Sterol regulatory element binding transcription factor 1 promotes proliferation and migration in head and neck squamous cell carcinoma. PeerJ. 2023;11: e15203. [DOI] [PubMed] [PMC]