RESEARCH ARTICLE

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# Exploring the Role and Prognostic Value of *TMEM208* in Head and Neck Squamous Cell Carcinoma

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## Abstract

**Objective:** Head and neck squamous cell carcinoma (HNSCC) is one of the world's eight most common malignancies and a severe hazard to human health. Transmembrane protein 208 (*TMEM208*) has been reported to be associated with autophagy,which is strongly tied to the onset and development of numerous illnesses, including cancer. For this reason, we investigated the expression and prognostic significance of *TMEM208* in HNSCC. **Methods:** To explore the role and molecular mechanism of this gene in HNSCC, we performed a comprehensive analysis of the *TMEM208* gene, including gene expression analysis, prognostic analysis, and immune infiltration analysis using the UALCAN, HPA, CVCDAP, DAVID, TIMER, CIBERSORTx, TISIDB, and cBioPortal online databases. It was further validated by in vitro cell culture. **Results:** Analysis of TCGA data showed that *TMEM208* was highly expressed in HNSCC ($P < 0.01$) and significantly correlated with several clinicopathologic features, and in vitro cellular assays demonstrated that *TMEM208* was highly expressed in multiple squamous carcinoma cell lines. Survival analysis showed that high expression of *TMEM208* decreased OS (P=0.0088), PFI (P=0.0062), and DSS (P=0.0036) in HNSCC patients. cox regression analysis indicated that high expression of *TMEM208* was an independent risk factor for OS in HNSCC patients ($P<0.05$). In addition, functional enrichment analysis showed that *TMEM208* was closely associated with translation, ribosomal and mitochondrial functions, and GSVA analysis showed that *TMEM208* was negatively correlated with a variety of immune cell differentiation in HNSCC, with a statistically significant difference. Immunocorrelation analysis showed that *TMEM208* could affect immune cell infiltration in HNSCC; in addition, *TMEM208* correlated with CD24, CD276, LAG3, and HVEM. **Conclusion:** In conclusion, *TMEM208* holds promise as a prognostic indicator for HNSCC and is closely related to ICI treatment.

**Keywords:** *TMEM208*- prognostic biomarkers- immune checkpoints- head and neck squamous cell carcinoma

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## Introduction

Head and neck squamous cell carcinoma (HNSCC)-one of the most prevalent malignant tumors takes into account more than 95% of head and neck cancers and ranks eighth among all cancers in terms of mortality rate [1, 2]. Most HNSCCs develop from epithelial tissue in the mouth and pharynx,and they are usually caused by irritants, such as prolonged cigarette smoking, alcohol consumption, and betel nut chewing [3]. In the early stages, metastasis to surrounding tissues and distant organs often occurs through lymphatic channels. Patients have a poor prognosis because of the failure of immediate treatment and complete removal of metastatic foci; the rate of survival over a 5-year period for patients with advanced disease is often under 30% [4]. Common treatments for HNSCC include surgical treatment, chemotherapy, radiotherapy, and combination therapy [3]. With the deepening of research, immunotherapy, especially immune checkpoint inhibitors (ICI), has gradually entered the public eye and become an important part of treatment [5].

ICI therapies, such as those targeting PD-L1, PD1, and CTLA4, have significantly increased the overall survival time (OS) of patients with refractory, recurrent, and metastatic HNSCC compared with conventional treatments [6]. Currently, ICI therapy is used in the first-line treatment of relapsed and metastatic (R/M) HNSCC as well as in the second-line treatment of platinum-refractory R/M HNSCC [7, 8]. In addition, the use of ICI in neoadjuvant therapy is being actively investigated [9-11]. However, ICI can only benefit some patients, and in-depth analysis and focused research on the immune architecture of HNSCC patients undergoing ICI remain crucial due to the low response rate to its treatment in most patients. Recently, many novel immunotherapies, including CAR-T cell therapy, lysosomal virus therapy, and vaccines, are also being studied [12]. Therefore, the identification of valuable biomarkers to predict the efficacy of immunotherapy for HNSCC is warranted [13].

Autophagy is an organism's defense mechanism

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that maintains normal physiological functions by removing aged, necrotic, and diseased tissues. Autophagy dysregulation is often associated with many diseases, which includes degenerative diseases, infectious diseases, cardiomyopathies, and malignancies [14, 15]. Autophagy-associated proteins are crucial in cancers as a type of programmed cell death. In cancer development, autophagy plays a role in inhibiting carcinogenesis by removing damaged tissues and oncogenic-associated proteins production; however, in advanced stages of tumors, autophagy can promote tumor metastasis and progression by preserving tumor cell functions (e.g., maintenance of mitochondrial function, reduction of DNA damage, and enhancement of cancer cell stress capacity) [16, 17]. reported that autophagy could be involved in chemoresistance in glioblastoma. Zhu et al (2021) [18] demonstrated that autophagy repression could accelerate the malignant progression of nasopharyngeal carcinoma. Transmembrane protein 208 (*TMEM208*) has been reported to be associated with autophagy and can inhibit autophagy when overexpressed and expression inhibition can promote autophagy [19]. According to Zhu et al (2017) [20]., *TMEM208* and PQLC2 are suitable molecular targets for aspirin detection in colon cancer.

Therefore, the current work used bioinformatics methods to comprehensively assess the link between *TMEM208* expression and HNSCC prognosis and further investigate the interaction between tumor immune cell infiltration and *TMEM208* expression. These results will provide new insights into the function of *TMEM208* and the treatment of HNSCC.

## Materials and Methods

*Data collection and processing*

The TCGA (http://portal.gdc.cancer.gov) database was used to obtain clinical and transcriptomic data from HNSCC patients. The TCGA database is a publicly funded project that provides gene expression data and sequencing data for a wide range of cancers, as well as patient-specific clinical data [21]. A total of 566 specimens were obtained from the TCGA database, of which 44 were normal. Clinical information was present in 521 patients, and patients with incomplete clinical information were deleted when relevant analyses were performed.

*Expression analysis*

The differential expression of *TMEM208* in cancerous and normal tissues, as well as under various clinicopathological features, was analyzed using R software. The UALCAN platform (http://ualcan.path.uab.edu) was used to supplement the association between *TMEM208* expression and relevant clinicopathological parameters in HNSCC patients. UALCAN is an easy-to-use interactive platform based on the TCGA database [22]. It can be utilized to analyze and query the correlation between tumor stage, grading, race, and other relevant clinicopathological features and gene expression.

*Immunohistochemical analysis*

The Human Protein Atlas (HPA)(https://www.proteinatlas.org/) database was applied to acquire immunohistochemical staining data for the distribution of *TMEM208* protein expression in normal mouth epithelium and malignant tissues. The antibody for *TMEM208* immunohistochemical staining was obtained from Sigma-Aldrich (HPA041949).

*Survival analysis*

CVCDAP (https://omics.bicancer.org/cvcdap/) is a Webde1-based online open platform that provides customizable and interactive toolboxes for the analysis of TCGA and CVCDAP public datasets as well as user-defined uploaded data (Guan et al, 2020) [23]. This study analyzed the link between *TMEM208* expression and the survival of HNSCC patients dependent on the CVCDAP online platform. The median *TMEM208* mRNA expression level was utilized to categorize patients into higher and lower expression groups; statistical significance was determined using P-value and risk ratio (HR).

*Univariate and Multivariate Cox analyses*

Proportional hazards model analyses were used to evaluate the association of age, sex, clinicopathologic factors, and OS with *TMEM208* expression. Based on *TMEM208* expression, this analysis categorized *TMEM208* into high-expression and low-expression groups. We deleted two patients who lacked OS data.

*Enrichment analysis*

DAVID (https://david.ncifcrf.gov.) is a comprehensive functionally annotated database that, for a given list of genes, is capable of performing a variety of functional analyses, such as gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. We used the downloaded dataset for Pearson analysis, setting R greater than 0.5 and P less than 0.05 as the inclusion criteria, followed by functional enrichment and signaling pathway analyses in DAVID, respectively. Based on the gene lists in the GSEA (https://www.gsea-msigdb.org) database, we conducted gene set variation analysis (GSVA) to better understand the role of *TMEM208*. R was used to determine the degree of connection between each gene list's functional enrichment scores and *TMEM208* expression.The p-value's size reveals the statistical significance of the correlation's degree.

*Analysis of the TIMER database*

TIMER (https://cistrome.shinyapps.io/timer/) is a public database, and for any given target gene, we can analyze its relationship with the level of immune cell infiltration. To understand the effect of *TMEM208* expression on tumor immunity, we used TIMER for analysis.

*Analysis of the cBioPortal database*

We used cBioPortal (https://www.cbioportal.org), a comprehensive data analysis platform for cancer genomics, to explore the mutation rate of *TMEM208* in HNSCC and the association of TMEM2058 with TP53, CDKN2A, FAT1, NOTCH1, KMT2D, NSD1, and TGFBR2 mutually exclusive [24-26].

*Analysis of the CIBERSORTx database*

An online analytic tool called CIBERSORTx (http://cibersort.stanford.edu) may be used to calculate gene expression profiles and calculate the relative abundance of various cell types in mixed cell populations (Newman et al 2019) [27]. In order to examine the abundance of 22 distinct immune cells in tissues, upload gene expression profiling data to CIBERSORTx and use the LM22 standard matrix file. This study used CIBERSORTx online to compare the proportion of 22 immune cells in cancer tissues between the groups with high and low *TMEM208* expression.

*Analysis of the TISIDB database*

The TISIDB (http://cis.hku.hk.) website, which contains multiple kinds of heterogeneous data, examines the relationships between immune systems and tumors [28] . The TISIDB database was utilized to support our analysis of the relationship between lymphocytes and *TMEM208* expression.

*RT-qPCR analysis*

In vitro mRNA expression validation was performed using human normal keratinocyte-forming cells (HOK) and head and neck squamous cell carcinoma cell lines (CAL27, SCC25 and SCC9). The cDNA was synthesized with HiScript IIQ RT SuperMix for qPCR Reagent Kit (Vazyme, China). All real-time PCR reactions were performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme,China). GAPDH (F:GACCTGA CCTGCCGTCTA,R:AGGAGTGGGGTGTCGCTGT) was used as a control to quantify and normalize the amplified PCR products.The primers for the *TMEM208* gene were F:TATCCTTCCGTGATCCTA and R: ATTCTGGTCTTCTTGGGT.

## Results

*TMEM208 is highly expressed in HNSCC*

We analyzed the expression of *TMEM208* in several malignant tumor tissues and discovered that HNSCC was one of the tumor tissues that *TMEM208* was substantially expressed in (Figure 1A). Using mRNA expression data from HNSCC patients in the TCGA database, our study revealed that *TMEM208* expression was noticeably increased in tumor tissues (Figure 1B), and the expression of *TMEM208* was also notably increased when contrasted with paired paracancerous tissues (Figure 1C). Immunohistochemical staining and in vitro cell line results were consistent with the analysis, with deepened cytoplasmic staining seen in cancer tissues (Figure1D) and high expression of *TMEM208* in squamous carcinoma cell lines (Fingure1E).

*TMEM208 expression and clinicopathologic features*

To explore the impact of *TMEM208* on HNSCC patients, we analyzed the relationship between *TMEM208* and clinicopathological characteristics (Figure 2). In HNSCC patients, *TMEM208* expression was significantly higher and statistically significant in the clinical grade, T-stage, N-stage and M-stage of the tumors compared with normal tissues. This funding suggests that *TMEM208* is a risk factor for HNSCC patients. Gender analysis showed that *TMEM208* was significantly highly expressed in male patients (Figure S1). Further analysis of the UALCAN database showed (Figure S1) that *TMEM208* was significantly highly expressed in HPV-negative patients and that Asians had significantly lower *TMEM208* expression relative to other ethnic groups.

*Association of TMEM208 expression with survival in HNSCC patients*

As shown in Figure 3, the OS, progression-free interval (PFI), and disease-specific survival (DSS) of patients with high *TMEM208* expression were relatively poor compared with those in the low expression group. This finding suggests that *TMEM208* can significantly affect the prognostic survival of patients and that *TMEM208*'s high expression shortens post-operative patient survival times.

*High TMEM208 expression is an independent predictor of HNSCC patients*

To explore the effect of *TMEM208* on the prognostic survival of HNSCC patients, we performed a prognostic proportional risk modeling analysis based on the TCGA database. In univariate analysis, age, gender, M stage, and high *TMEM208* expression, which were able to independently influence the prognosis of HNSCC patients (Table 1). In multifactorial analysis, age, M stage, and high *TMEM208* expression were adverse effects for the prognosis of HNSCC patients. Univariate and multifactorial analyses showed that high *TMEM208* expression could serve as an independent biomarker for HNSCC and was not associated with the age, gender, and M stage of HNSCC patients (Table 1).

*Genetic alteration of TMEM208 in HNSCC*

Using cBioPortal, we explored the genetic alterations of *TMEM208* in HNSCC and simultaneously explored the genetic alterations of TP53, CDKN2A, FAT1, NOTCH1, KMT2D, NSD1, and GFBR2 under the same sample data (530 cases) (Figure 4). We found that *TMEM208* alterations in HNSCC were mainly amplification and deep deletion, with a genetic change of 0.8%. Mutual exclusion analysis revealed a strong co-expression relationship between *TMEM208* and TGFBR2 in HNSCC (Table S1).

*TMEM208 enrichment analysis*

We used a Pearson correlation analysis to filter the group of genes most closely related to *TMEM208* in order to investigate the biological processes linked with this gene. The inclusion criteria were |R| larger than 0.5 and P-value less than 0.05. This study performed GO and KEGG analyses based on the aforementioned gene sets. The most relevant biological processes to *TMEM208* were translation, cytoplasmic translation, and mitochondrial ATP synthesis coupled proton transport (Figure 5A). Analysis of cellular fractions indicated that *TMEM208* is primarily located in the mitochondrial inner membrane and ribosome (Figure 5C). Molecular functions are the structure of ribosomes, RNA binding, and protein binding (Figure 5B). The main signaling pathways of *TMEM208*

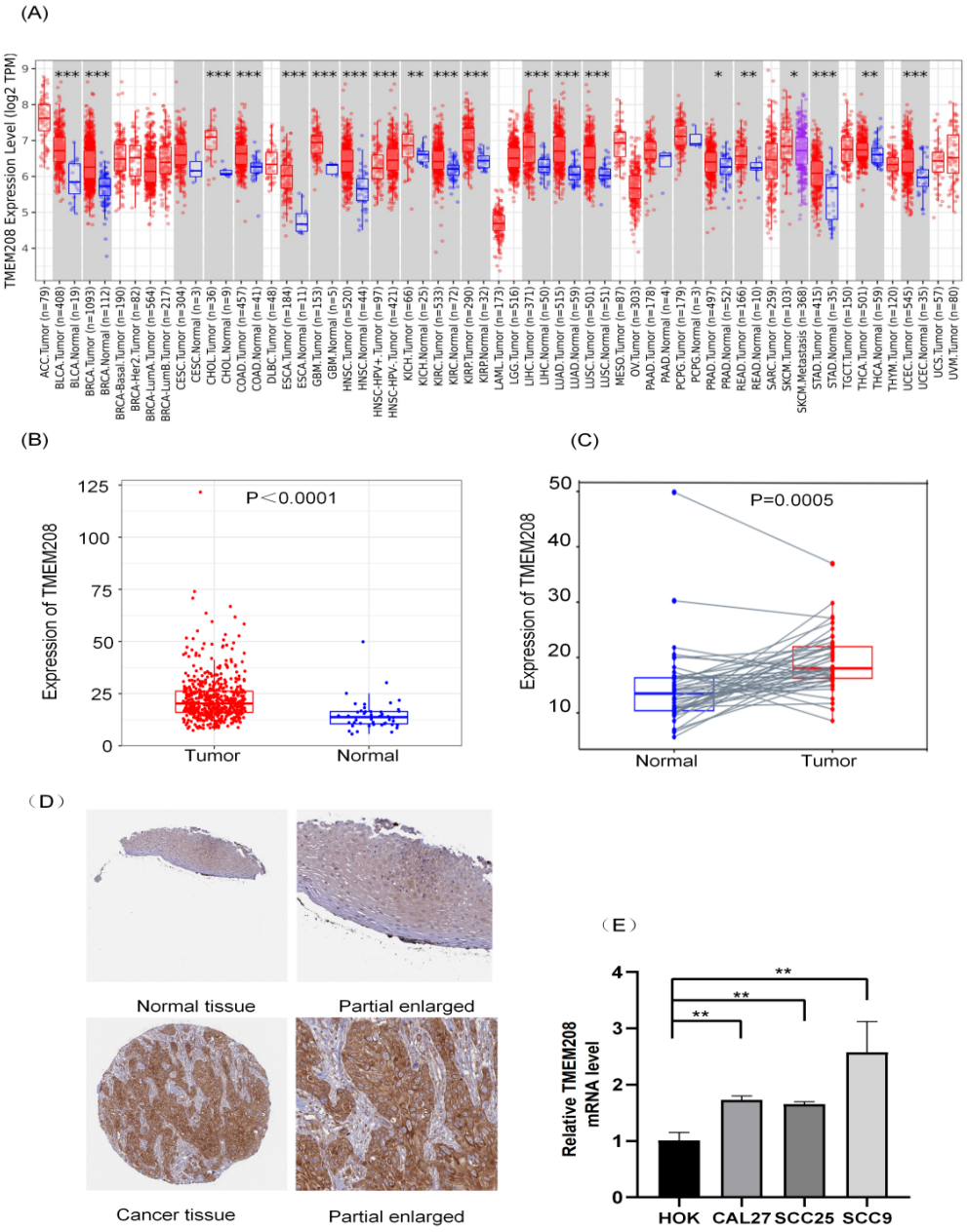


Figure 1. Analysis of the Expression of *TMEM208*. (A) Expression levels of *TMEM208* in different tumors. (B) Expression of TMEM208 in healthy and malignant tissues is compared. (C) Expression analysis of TMEM208 in paired samples. (D) Immunohistochemical pictures under HPA041949 antibody staining. (E) mRNA expression of *TMEM208* in oral keratin-forming cells (HOK) and CAL27, SCC25 and SCC9 cell lines (** P < 0.01). The significance of the difference was tested using an unpaired t test.

include ribosomals and oxidative phosphorylation (Figure 5D). These findings suggest that *TMEM208* on HNSCC cells may play a significant role in carcinogenesis and development.

To further understand the impact of *TMEM208* in HNSCC, we employed gene set variation analysis (GSVA) (Figure 6). According to enrichment findings and correlation analysis, *TMEM208* expression in HNSCC had a positive relationship with ribosomal and mitochondrial functions, as well as ATP biosynthesis (Figures 6(A)), and was negatively correlated with immune system activation and the differentiation of numerous immune cells (Figure 6(B)). These findings suggest an association between *TMEM208* and the vital activities of tumor cells and immunosuppression.

*TMEM208 expression and tumor immune infiltration*

We sorted the samples into high and low expression groups based on *TMEM208* expression and then utilized CIBERSORTx, an online analytic tool, to investigate the relationship between the *TMEM208* transcript and the 22 immune cells offered by the CIBERSORTx system. Among them, the degree of infiltration of B cell naive, T cell CD4 memory resting, NK cells resting, NK cells activated, and Neutrophils was significantly reduced in the high expression group compared with the low expression group.The results show that when *TMEM208* expression increases, the abundance of immune cells

Table 1.Univariate and Multivariate Analysis of Clinicopathologic Factors and *TMEM208* Expression

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| Variables | P-value | HR | 95% CI | P-value | HR | 95% CI |
| Age | 0.001 | 1.022 | 1.009-1.034 | 0.003 | 1.019 | 1.006-1.033 |
| Gender | 0.036 | 0.736 | 0.553-0.980 | 0.176 | 0.812 | 0.601-1.098 |
| M stage | 0.039 | 2.84 | 1.053-7.662 | 0.043 | 2.786 | 1.032-7.519 |
| N stage | 0.084 | 1.136 | 0.983-1.314 | | | |
| T stage | 0.221 | 1.091 | 0.949-1.256 | | | |
| Grade | 0.272 | 1.088 | 0.936-1.265 | | | |
| Smoke | 0.982 | 0.997 | 0.761-1.306 | | | |
| Alcohol | 0.648 | 0.936 | 0.705-1.244 | | | |
| *TMEM208* (low VS high) | 0.018 | 1.38 | 1.057-1.802 | 0.028 | 1.354 | 1.033-1.774 |

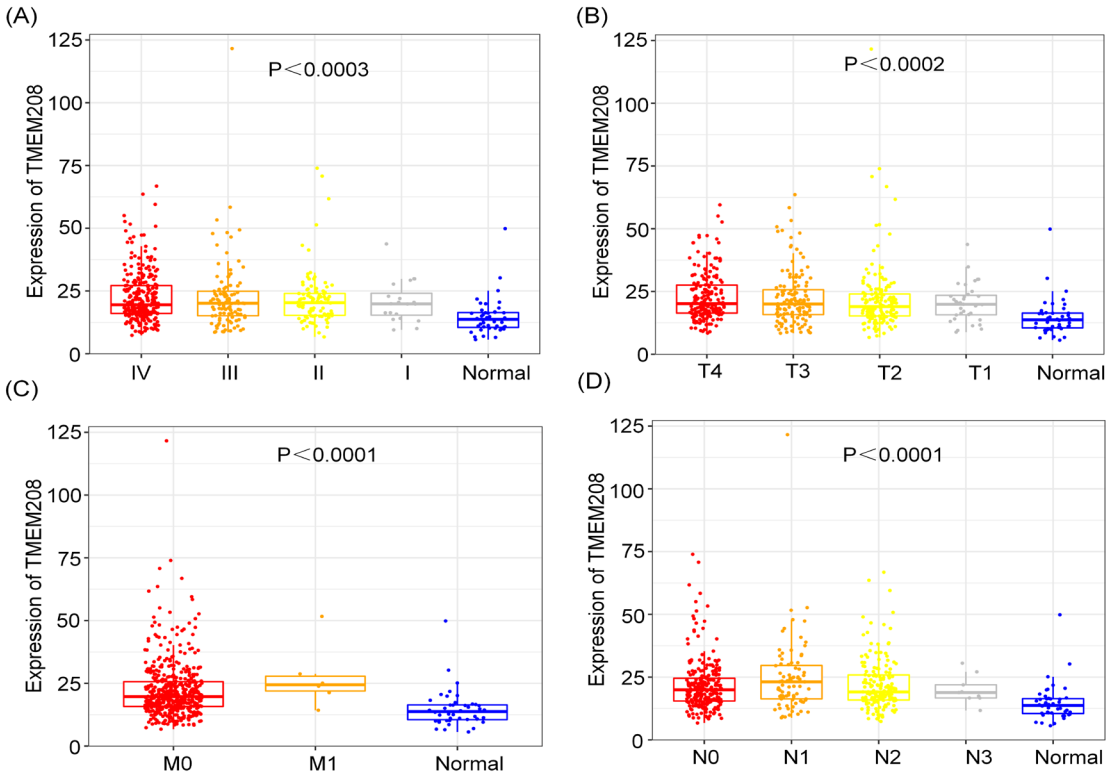


Figure 2. High *TMEM208* Expression was Associated with Poor Patient Prognosis. (A) Tumor clinical grading, (B) Tumor clinical T-stage, (C) Tumor clinical M-stage, (D) Tumor clinical N-stage. Statistical differences were the result of a one-way ANOVA.

reduces (Figure 7).

Using the TIMER and the TISIDB databases, we further investigated the impact of *TMEM208* transcript levels on anti-tumor immune cells. We found that *TMEM208* expression was negatively correlated with the infiltration of numerous immune cells such as B cells, CD8+T, CD4+T, neutrophils, dendritic cells, T follicular helper cells, NK cells, NKT cells, and mast cells (Figures 8, Figures S2). Correlation analysis showed (Table 2) that *TMEM208* expression was positively correlated with CD24, CD276, LAG3 and HVEM immune checkpoints. These fundings imply that *TMEM208* is critical for the suppression of immunological infiltration in the immune microenvironment of HNSCC.

Table 2.Relationship between *TMEM208* Expression and Immune Checkpoints

| | CD24 | CD276 | LAG3 | HVEM |
|---|---|---|---|---|
| R | 0.131059 | 0.144055 | 0.102806 | 0.111103 |
| P value | 0.002699 | 0.000965 | 0.018801 | 0.011079 |

## Discussion

HNSCC is the most common and high mortality cancer of the head and neck [29]. Conventional treatments (usually surgical resection, radiotherapy, and systemic therapy) have failed to achieve satisfactory overall results in HNSCC patients [30, 31]. Although patients with many cancers benefit from immunotherapy, the

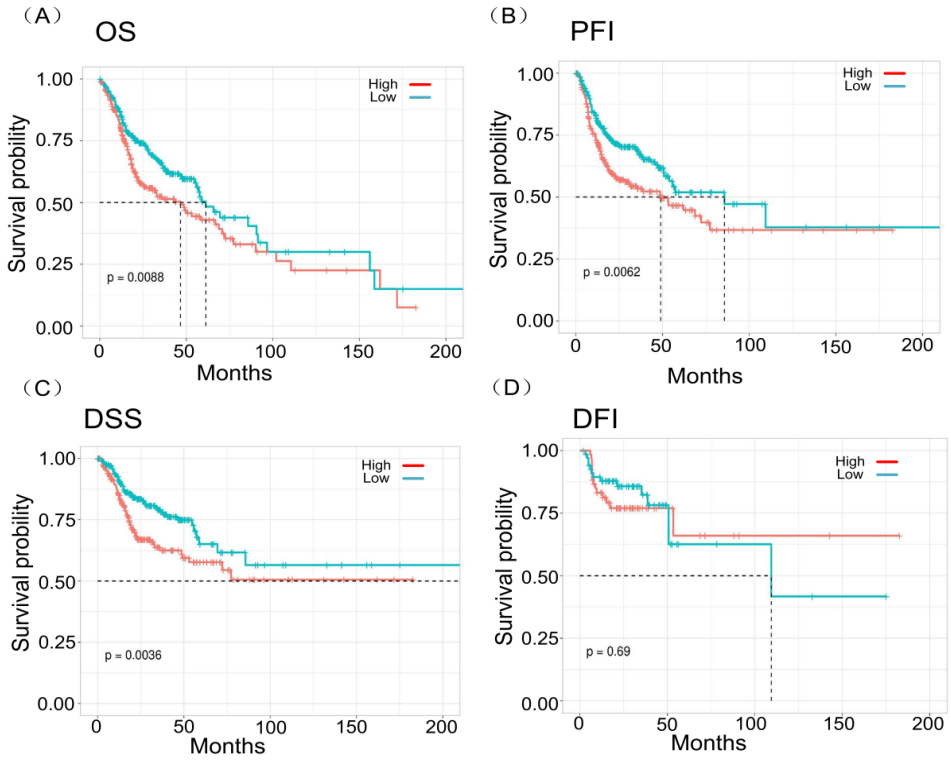


Figure 3. Relationship between *TMEM208* and Survival of HNSCC Patients. The effects of *TMEM208* expression on different survival times of HNSCC patients are shown in the figures, respectively.

clinical outcomes of immunotherapy in HNSCC patients remain disappointing [32, 33]. Therefore, it is crucial to find biomarkers associated with the development and progression of HNSCC, especially molecular targets that are relevant for immunotherapy. Recently, *TMEM208* has been shown to associate with cellular autophagy, and dysregulation of autophagy has been linked to many diseases, including cancer [34]. Therefore, our study evaluates the role and prognostic impact of *TMEM208* on HNSCC. We found that *TMEM208* was highly expressed in various tumor tissues, such as ESCA, LUSC, and HNSCC. The TCGA database's study of *TMEM208* mRNA-seq data revealed that this gene was considerably overexpressed in HNSCC tissues, and paired-tissue analysis revealed that the expression of this gene was greater in tumor tissues than in paracancerous tissues. Immunohistochemistry and in vitro cell line analysis further confirmed the results. We discovered that *TMEM208* was connected with many pathological aspects by investigating the association between *TMEM208* expression and clinicopathological elements.

Using CVCDAP online database analysis, we discovered that the expression level of *TMEM208* significantly impacted the prognosis of HNSCC patients. High *TMEM208* expression significantly reduced patients' prognostic survival time, including OS, DFI, and DSS. High expression of *TMEM208* in HNSCC patients was shown by the findings of univariate and multivariate

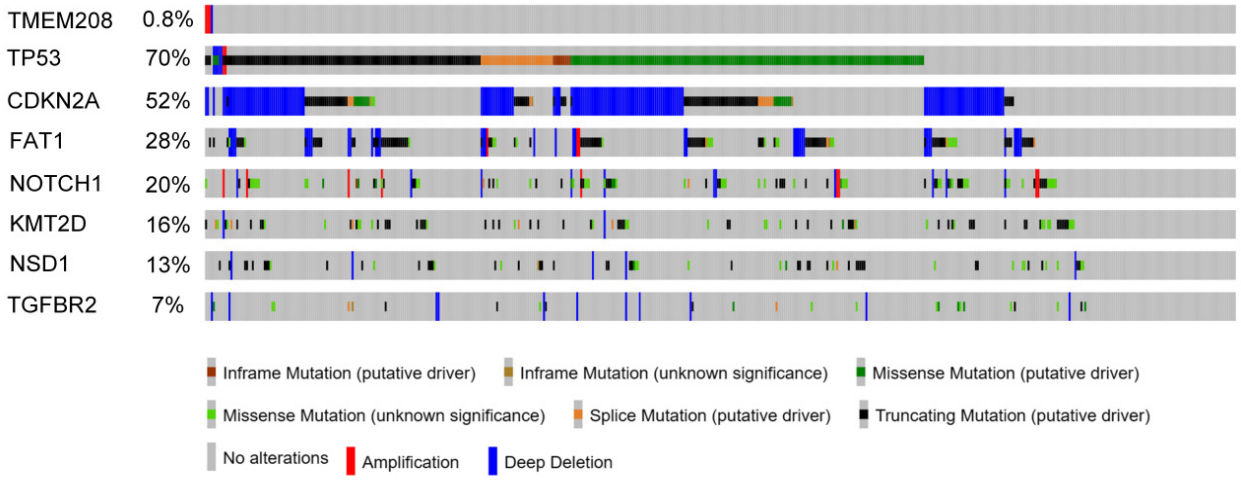


Figure 4. Mutation Analysis of *TMEM208* and *TGFBR2, TP53, NOTCH1, KMT2D, NSD1, FAT1, CDKN2A* in HNSCC.

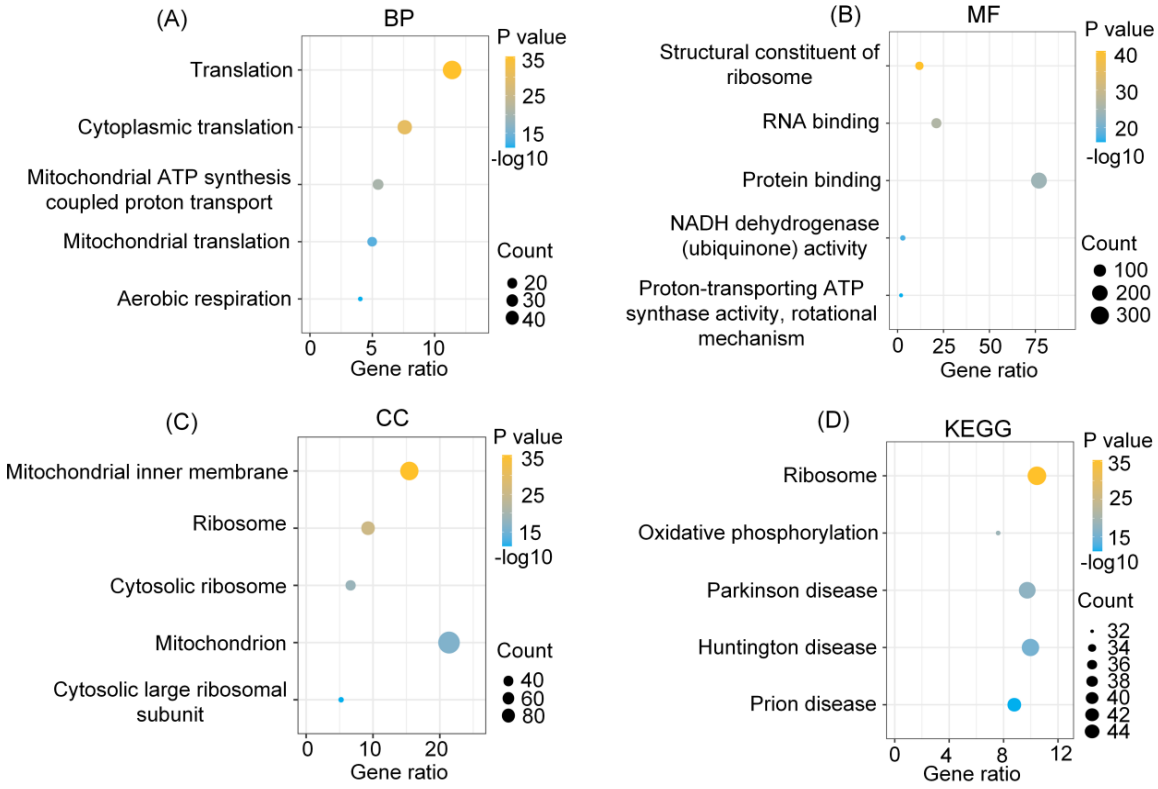


Figure 5.*TMEM208* is Closely associated with HNSCC Development. (A) biological process (BP), (B) molecular function (MF), (C) cellular component (CC), and (D) Kyoto Encyclopedia of Genes and Genes (KEGG).

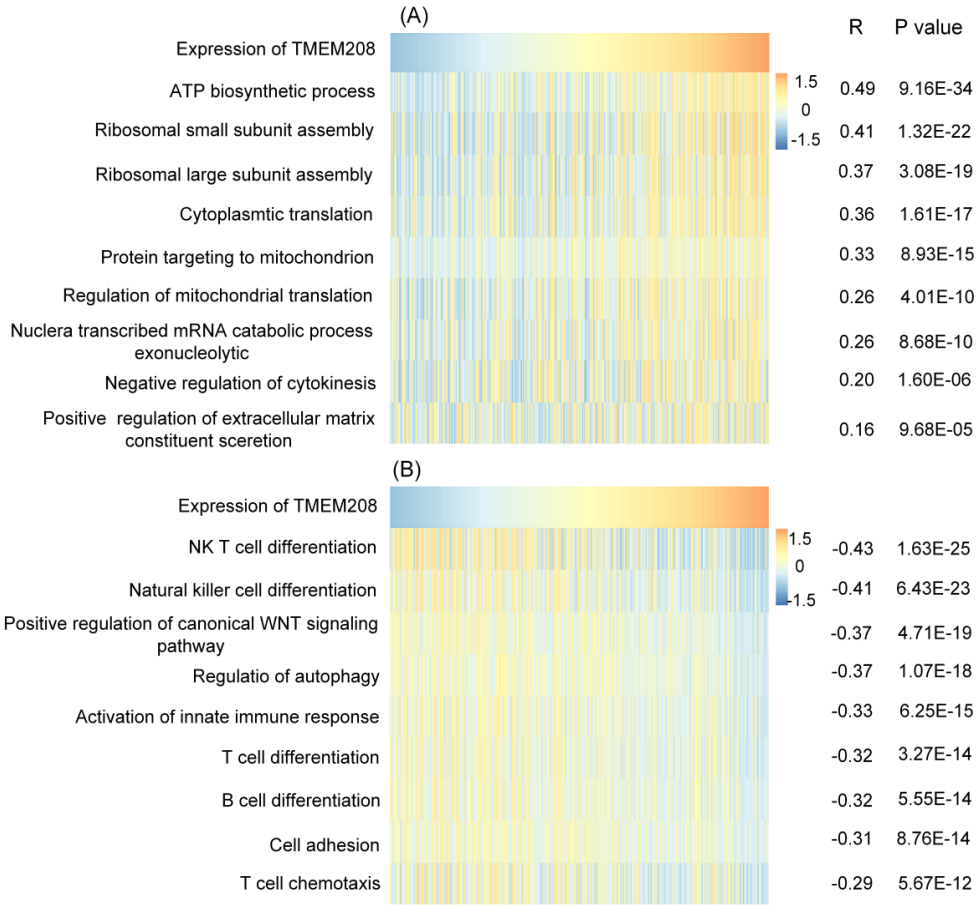


Figure 6. GSVA Analysis of the *TMEM208* Expression. (A) Heatmap showing functional enrichment scores for positive correlation between *TMEM208* expression and each patient in The Cancer Genome Atlas (TCGA) database, (B) functional enrichment scores for negative correlation with *TMEM208* expression. From left to right ,TMEM208 expression is elevated. The correlation was tested by Pearson correlation analysis.

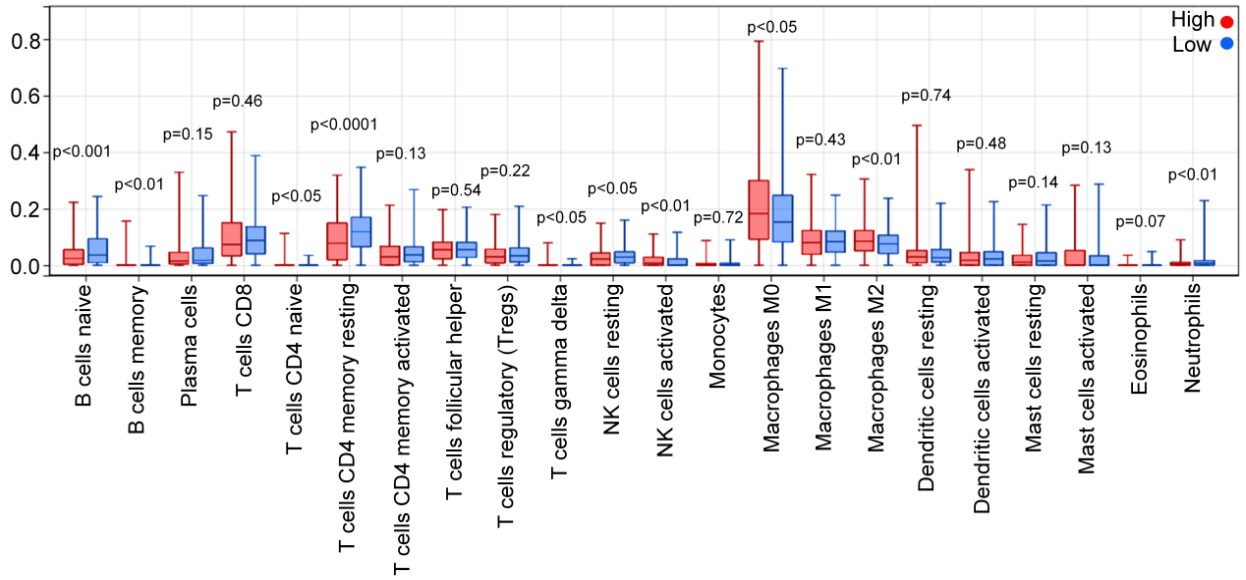


Figure 7. *TMEM208* Expression and Immune Infiltration in HNSCC Samples. The significance of the difference was tested by Wilcoxon test.

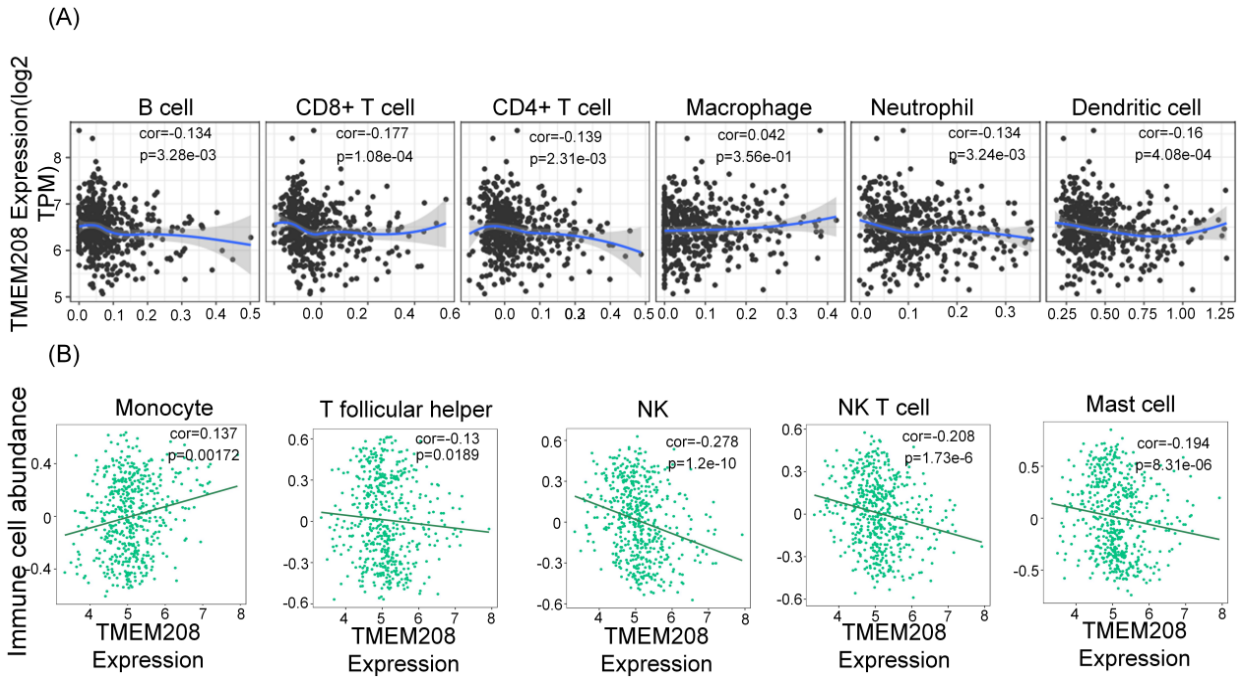


Figure 8. Relationship between *TMEM208* Expression Level and Immune Cell Infiltration.

analyses to be an independent predictor of prognosis. In this analysis, gender was a favorable factor for HNSCC patients. The cBioPortal analysis showed a degree of genetic alteration in *TMEM208* and co-occurrence with TGFBR2 in HNSCC. Therefore, *TMEM208* has potential diagnostic value for HNSCC patients.

To understand the oncogenic mechanism of *TMEM208* in HNSCC, we performed a functional enrichment analysis. In HNSCC, *TMEM208* biological processes are associated with translation, cytoplasmic translation, mitochondrial translation, and mitochondrial ATP synthesis [35-37]. Abnormal mRNA translation accelerates cancer progression and increases stress adaptation in cancer cells, whereas the synthesis of large amounts of proteins inevitably requires a greater supply of ATP [38]. Adequate energy supply further promotes tumor development. In terms of cellular components, *TMEM208* is closely related to ribosomes and mitochondria. Increased ribosome number is associated with abnormal ribosome occurrence and changes in nucleolus size and number, whereas aberrant rRNA synthesis and dysregulation of ribosomal proteins are strongly associated with cancer development [39, 40]. Apoptosis and programmed cell death are mostly regulated by the mitochondria [41], and abnormal mitochondrial activity and impaired apoptosis are indicators of cellular carcinogenesis [42].

This finding suggests that *TMEM208* potentially affects tumorigenesis and development in HNSCC. Further GSVA analysis showed that *TMEM208* expression was positively correlated with ATP biosynthesis and the function of ribosomes and mitochondria and negatively correlated with the activation of the immune system and the differentiation and cell adhesion of immune cells [43], suggesting that *TMEM208* can mediate tumor immunomodulation and has a promotional effect on tumor metastasis.

CIBERSORTx analysis showed that *TMEM208* expression significantly affected multiple immune cell infiltrates in HNSCC patients. *TMEM208* expression was found to be negatively connected with the infiltration of a variety of immune cells in the correlation study with immune cells, including B cells, CD8+T, CD4+T, neutrophils, dendritic cells, NK cells, and mast cells. Among these, an increase in CD8+T and CD4+T is linked to a favorable prognosis, particularly CD8+T, which is a crucial component of the body's anti-tumor immune system, and an increase in NK cells leads to better patient outcomes [44, 45]. CD276 and LAG-3 can inhibit T cell proliferation and activation [1] [46]; CD24 inhibits macrophage phagocytosis of tumors [47]; HVEM is widely present with T cells, B cells, and NK cells, which can produce both immunosuppressive and immune-activating signals [48]. Immune infiltration and immune checkpoint correlation analysis showed a potential correlation between *TMEM208* expression and immunosuppression. Therefore, *TMEM208* may have an important role in immune escape from HNSCC.

Our research showed that *TMEM208* regulates the tumor immune microenvironment of HNSCC and actively promotes the growth and progression of HNSCC through many different kinds of mechanisms. However,our study has a few limitations. First, our study is an analysis using the data in the database, and its actual relevant results must be verified using further experiments. Second, the role of *TMEM208* on tumor cells and immune infiltrating cells and how it affects the prognosis of HNSCC patients-related molecular mechanisms must be further explored.

In conclusions, in this study, we found that *TMEM208* was significantly highly expressed in HNSCC ($P < 0.01$) and was associated with poorer OS in patients. Age, tumor M stage and high expression of *TMEM208* were independent risk factors for HNSCC. In addition, *TMEM208* contributed to the development of HNSCC by affecting mitochondrial and ribosomal functions and played an important role in the immune escape of tumor cells by affecting the infiltration of multiple immune cells (B-cells, CD+8T, CD4+T, etc.) and the differentiation of immune cells (NK T-cells, T-cells, B-cells). And *TMEM208* was co-expressed with known HNSCC mutant genes. Therefore, we believe that *TMEM208* may be a potential biomarker for HNSCC and may have an important role in tumor ICI therapy.

## Author Contribution Statement

Jing Yang contributed to study design, performed data analysis, interpreted the results, and drafted the manuscript; Tao Song project supervision and reviewed and revised the manuscript.

## Acknowledgements

*Data Availability*

All publicly accessed data are available on databases described in methodology. Analyzed datasets used during the study can be made available from the corresponding author upon reasonable request.

*Human and animal rights*

No animals/humans were used for studies that are base of this research.

*Conflict of interest*

The authors declare no conflicts of interest.

## References

1. Mei Z, Huang J, Qiao B, Lam AK-y. Immune checkpoint pathways in immunotherapy for head and neck squamous cell carcinoma. Int J Oral Sci. 2020;12(1):16.
2. Cramer JD, Burtness B, Le QT, Ferris RL. The changing therapeutic landscape of head and neck cancer. Nat Rev Clin Oncol. 2019;16(11):669-83. https://doi.org/10.1038/s41571-019-0227-z.
3. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Author correction: Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2023;9(1):4. https://doi.org/10.1038/s41572-023-00418-5.
4. Chow LQ. Head and neck cancer. New England Journal of Medicine. 2020;382(1):60-72.
5. Sahu M, Suryawanshi H. Immunotherapy: The future of cancer treatment. J Oral Maxillofac Pathol. 2021;25(2):371. https://doi.org/10.4103/0973-029X.325257
6. Cramer JD, Burtness B, Ferris RL. Immunotherapy for head and neck cancer: Recent advances and future directions. Oral oncology. 2019;99:104460. https://doi.org/10.1016/j.oraloncology.2019.104460
7. Cohen EEW, Bell RB, Bifulco CB, Burtness B, Gillison ML, Harrington KJ, et al. The society for immunotherapy of cancer consensus statement on immunotherapy for the treatment of squamous cell carcinoma of the head and neck (hnscc). J Immunother Cancer. 2019;7(1):184. https://doi.org/10.1186/s40425-019-0662-5.
8. Ferris RL, Haddad R, Even C, Tahara M, Dvorkin M, Ciuleanu TE, et al. Durvalumab with or without tremelimumab in patients with recurrent or metastatic head and neck squamous cell carcinoma: Eagle, a randomized, open-label phase iii study. Ann Oncol. 2020;31(7):942-50. https://doi.org/10.1016/j.annonc.2020.04.001.
9. Liu J, Blake SJ, Yong MC, Harjunpää H, Ngiow SF, Takeda K, et al. Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discov. 2016;6(12):1382-99. https://doi.org/10.1158/2159-8290.Cd-16-0577.
10. Liu J, O'Donnell JS, Yan J, Madore J, Allen S, Smyth MJ, Teng MWL. Timing of neoadjuvant immunotherapy in relation to surgery is crucial for outcome. Oncoimmunology. 2019;8(5):e1581530. https://doi.org/10.1080/2162402x.2019.1581530.
11. Friedman J, Moore EC, Zolkind P, Robbins Y, Clavijo PE, Sun L, et al. Neoadjuvant pd-1 immune checkpoint blockade

reverses functional immunodominance among tumor antigen-specific t cells. Clin Cancer Res. 2020;26(3):679-89. https://doi.org/10.1158/1078-0432.Ccr-19-2209.
12. Parmar K, Mohamed A, Vaish E, Thawani R, Cetnar J, Thein KZ. Immunotherapy in head and neck squamous cell carcinoma: An updated review. Cancer Treat Res Commun. 2022;33:100649. https://doi.org/10.1016/j.ctarc.2022.100649.
13. Chen Y, Li ZY, Zhou GQ, Sun Y. An immune-related gene prognostic index for head and neck squamous cell carcinoma. Clin Cancer Res. 2021;27(1):330-41. https://doi.org/10.1158/1078-0432.Ccr-20-2166.
14. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069-75. https://doi.org/10.1038/nature06639.
15. Onorati AV, Dyczynski M, Ojha R, Amaravadi RK. Targeting autophagy in cancer. Cancer. 2018;124(16):3307-18. https://doi.org/10.1002/cncr.31335
16. Li X, He S, Ma B. Autophagy and autophagy-related proteins in cancer. Mol Cancer. 2020;19(1):12. https://doi.org/10.1186/s12943-020-1138-4.
17. Hombach-Klonisch S, Mehrpour M, Shojaei S, Harlos C, Pitz M, Hamai A, et al. Glioblastoma and chemoresistance to alkylating agents: Involvement of apoptosis, autophagy, and unfolded protein response. Pharmacol Ther. 2018;184:13-41. https://doi.org/10.1016/j.pharmthera.2017.10.017.
18. Zhu Q, Zhang Q, Gu M, Zhang K, Xia T, Zhang S, et al. Mir106a-5p upregulation suppresses autophagy and accelerates malignant phenotype in nasopharyngeal carcinoma. Autophagy. 2021;17(7):1667-83. https://doi.org/10.1080/15548627.2020.1781368.
19. Zhao Y, Hu J, Miao G, Qu L, Wang Z, Li G, et al. Transmembrane protein 208: A novel er-localized protein that regulates autophagy and er stress. PLoS One. 2013;8(5):e64228. https://doi.org/10.1371/journal.pone.0064228.
20. Zhu Y, Yang C, Weng M, Zhang Y, Yang C, Jin Y, et al. Identification of tmem208 and pqlc2 as reference genes for normalizing mrna expression in colorectal cancer treated with aspirin. Oncotarget. 2017;8(14):22759-71. https://doi.org/10.18632/oncotarget.15191.
21. Tomczak K, Czerwińska P, Wiznerowicz M. The cancer genome atlas (tcga): An immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19(1a):A68-77. https://doi.org/10.5114/wo.2014.47136.
22. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S. Ualcan: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017;19(8):649-58. https://doi.org/10.1016/j.neo.2017.05.002.
23. Guan X, Cai M, Du Y, Yang E, Ji J, Wu J. Cvcdap: An integrated platform for molecular and clinical analysis of cancer virtual cohorts. Nucleic Acids Res. 2020;48(W1):W463-w71. https://doi.org/10.1093/nar/gkaa423.
24. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333(6046):1157-60. https://doi.org/10.1126/science.1208130.
25. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517(7536):576-82. https://doi.org/10.1038/nature14129.
26. Lu SL, Herrington H, Reh D, Weber S, Bornstein S, Wang D, et al. Loss of transforming growth factor-beta type ii receptor promotes metastatic head-and-neck squamous cell carcinoma. Genes Dev. 2006;20(10):1331-42. https://doi.org/10.1101/gad.1413306.
27. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol. 2019;37(7):773-82. https://doi.org/10.1038/s41587-019-0114-2.
28. Szkanderová S, Port M, Stulík J, Hernychová L, Kasalová I, Van Beuningen D, Abend M. Comparison of the abundance of 10 radiation-induced proteins with their differential gene expression in l929 cells. Int J Radiat Biol. 2003;79(8):623-33. https://doi.org/10.1080/09553000310001606821.
29. Mandal R, Şenbabaoğlu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight. 2016;1(17):e89829. https://doi.org/10.1172/jci.insight.89829.
30. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6(1):92.
31. Lacas B, Carmel A, Landais C, Wong SJ, Licitra L, Tobias JS, et al. Meta-analysis of chemotherapy in head and neck cancer (mach-nc): An update on 107 randomized trials and 19,805 patients, on behalf of mach-nc group. Radiother Oncol. 2021;156:281-93. https://doi.org/10.1016/j.radonc.2021.01.013.
32. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008;359(11):1116-27. https://doi.org/10.1056/NEJMoa0802656.
33. León X, Hitt R, Constenla M, Rocca A, Stupp R, Kovács AF, et al. A retrospective analysis of the outcome of patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck refractory to a platinum-based chemotherapy. Clin Oncol (R Coll Radiol). 2005;17(6):418-24. https://doi.org/10.1016/j.clon.2005.02.014.
34. White E. The role for autophagy in cancer. J Clin Invest. 2015;125(1):42-6.
35. Song P, Yang F, Jin H, Wang X. The regulation of protein translation and its implications for cancer. Signal Transduct Target Ther. 2021;6(1):68.
36. Morita M, Gravel SP, Hulea L, Larsson O, Pollak M, St-Pierre J, Topisirovic I. Mtor coordinates protein synthesis, mitochondrial activity and proliferation. Cell Cycle. 2015;14(4):473-80. https://doi.org/10.4161/15384101.2014.991572.
37. Porporato PE, Filigheddu N, Pedro JMB, Kroemer G, Galluzzi L. Mitochondrial metabolism and cancer. Cell Res. 2018;28(3):265-80. https://doi.org/10.1038/cr.2017.155.
38. Lee LJ, Papadopoli D, Jewer M, Del Rincon S, Topisirovic I, Lawrence MG, Postovit LM. Cancer plasticity: The role of mrna translation. Trends Cancer. 2021;7(2):134-45. https://doi.org/10.1016/j.trecan.2020.09.005.
39. Penzo M, Montanaro L, Treré D, Derenzini M. The ribosome biogenesis-cancer connection. Cells. 2019;8(1). https://doi.org/10.3390/cells8010055.
40. Pecoraro A, Pagano M, Russo G, Russo A. Ribosome biogenesis and cancer: Overview on ribosomal proteins. Int J Mol Sci. 2021;22(11). https://doi.org/10.3390/ijms22115496.
41. Wang C, Youle RJ. The role of mitochondria in apoptosis. Annu Rev Genet. 2009;43:95-118.
42. Kim HJ, Maiti P, Barrientos A. Mitochondrial ribosomes in cancer. Semin Cancer Biol. 2017;47:67-81. https://doi.org/10.1016/j.semcancer.2017.04.004.
43. Okegawa T, Pong RC, Li Y, Hsieh JT. The role of cell adhesion molecule in cancer progression and its application

in cancer therapy. Acta Biochim Pol. 2004;51(2):445-57.
44. Nguyen N, Bellile E, Thomas D, McHugh J, Rozek L, Virani S, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. Head Neck. 2016;38(7):1074-84. https://doi.org/10.1002/hed.24406.
45. Farlow JL, Brenner JC, Lei YL, Chinn SB. Immune deserts in head and neck squamous cell carcinoma: A review of challenges and opportunities for modulating the tumor immune microenvironment. Oral Oncol. 2021;120:105420. https://doi.org/10.1016/j.oraloncology.2021.105420.
46. Zhao B, Li H, Xia Y, Wang Y, Wang Y, Shi Y, et al. Immune checkpoint of b7-h3 in cancer: From immunology to clinical immunotherapy. J Hematol Oncol. 2022;15(1):153. https://doi.org/10.1186/s13045-022-01364-7.
47. Liu Y, Wang Y, Yang Y, Weng L, Wu Q, Zhang J, et al. Emerging phagocytosis checkpoints in cancer immunotherapy. Signal Transduct Target Ther. 2023;8(1):104. https://doi.org/10.1038/s41392-023-01365-z.
48. Ziogas DC, Theocharopoulos C, Lialios PP, Foteinou D, Koumprentziotis IA, Xynos G, Gogas H. Beyond ctla-4 and pd-1 inhibition: Novel immune checkpoint molecules for melanoma treatment. Cancers (Basel). 2023;15(10). https://doi.org/10.3390/cancers15102718.