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REVIEW ARTICLE

Gene mutation analysis and immune checkpoint therapy in head and neck squamous cell carcinoma*

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Abstract

Immune checkpoint inhibitors (ICI), represented by blocked programmed cell death-1 (PD-1), is a group of novel medicines for anti-tumor immunotherapy. It has been approved by the U.S. Food and Drug Administration (FDA) in recent years for relapsed or metastatic head and neck squamous cell carcinoma (HNSCC), and brings promising treatment prospects. However, the instability caused by tumor gene mutations significantly compromises the therapeutic effect of ICI. Therefore, the identification and analysis of HNSCC gene mutations can further guide and optimize the application of ICIs in HNSCC. In this study, we preliminarily described the clinical research progress of ICI therapy and the potential immune escape mechanism in HNSCC. An overview of complete HNSCC gene mutation results was generated from the bioinformatics study of TCGA database to further explain and analyze the relevant molecular mechanisms, which may aid in designing future personalized therapeutic strategies for HNSCC patients.

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Key words: head and neck squamous cell carcinoma (HNSCC); immune checkpoint inhibitor; gene mutation

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of malignant tumor worldwide. Although there has been significant progress recently in chemotherapy and adjuvant radiotherapy, there is no noticeable increase in the five-year survival rate among HNSCC patients, with metastasis and recurrence being one of the main causes for the poor prognosis and low survival rate of HNSCC patients [1]. In recent years, immunotherapy has gradually become a popular research topic because of its high effectiveness. Immune checkpoint inhibitors (ICIs), represented by blocking programmed cell death-1 (PD-1), have made breakthrough progress in the treatment of solid tumors such as lung cancer and melanoma, which not only reduces the efficiency of tumor metastasis and recurrence, but also effectively extends the survival time of patients [2–3]. Since 2016, two ICI therapies targeting relapsed and metastatic HNSCC, namely nivolumab and pembrolizumab, have been approved for marketing by the U.S. Food and Drug Administration (FDA). However, while the ICI therapeutic approach achieves better curative effects, drug resistance and serious adverse reactions were observed after long-term treatment. Consequently, the need to develop personalized treatment by adapting individual factors in HNSCC cases is enhanced. Recent studies have shown that the mutational gene phenotypes of cancer patients not only compromise the therapeutic efficiency of standardized drugs, but are also closely related to post-treatment adverse effects. Therefore, analysis of gene mutations in HNSCC patients can optimize individual therapy strategies and provide more precise and personalized ICI treatment plans [1, 4–6].

HNSCC and ICI treatment

Mechanism of HNSCC immune evasion

Although the immune evasion mechanism for HNSCC in the PD-1/PD-L1 pathway has not yet been determined, a literature review indicates that the mechanisms could be as follows [7–8]: 1. Induction of T cell apoptosis: The interaction between PD-L1 on the tumor surface and PD-1 from effector T cells causes either loss of T cell

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response or apoptosis, or both occur simultaneously; 2. By promoting immune tolerance, PD-L1 binds to CD80 on cytotoxic T cells to inhibit the immune response; 3. Via tumor cell activity regulation: Reverse transmission of the PD-L1 biological signaling pathway can prevent tumor cells from entering the apoptotic state; 4. Via inhibition of T cell proliferation, the PD-1/PD-L1 pathway inhibits PI3K/Akt/mTOR and Ras/MEK/Erk pathways, which further leads to the downregulation of amino acid and sugar metabolism, increase in fatty acid oxidation, enhancement of T cell differentiation, and induction of T cell depletion.

Clinical research of ICI treatment

The results of the Phase III trial (CheckMate 141) suggest that nivolumab treatment has better efficacy than standard treatment [9]. The results showed that the median overall survival (mOS) of the nivolumab group [7.5 months, 95% confidence interval (CI): 5.5-9.1] was significantly longer than the group receiving standard treatment (5.1 months, 95% CI: 4.0–6.0) (HR = 0.70; 97.73% CI: 0.51–0.96; P = 0.01). Another anti-PD-1 therapy, pembrolizumab, has also shown good results in a phase Ib clinical trial (KEYNOTE-012) in HNSCC patients. With an objective response rate (ORR) of 18% and median progression-free survival (mPFS) of 2 months, the mOS period was extended to 13 months [10]. A phase II clinical study (KEYNOTE-055) further reported the following results [11]: 16% of ORR, same mPFS (2.1 months), and a 2-month median response time. Nonetheless, the mOS outcome was shorter than that in the phase I record (8 months). Although further data from the phase III clinical trial (KEYNOTE-040) showed no significant prolongation of patients' OS with pembrolizumab treatment [12], the figures still indicate that patients with positive PD-L1 expression had better survival upon receiving pembrolizumab (mOS 11.6 months) than the low expression group (mOS 8.7 months), proving that PD-L1 can be used as an important prognostic factor for HNSCC patients upon ICI treatment. Furthermore, the identification and analysis of biomarker expression would benefit doctors in developing personalized ICI treatment strategies for HNSCC patients. However, it is worth noting that HNSCC patients treated with nivolumab and pembrolizumab both experienced adverse reactions such as fatigue, nausea, and loss of appetite. The records of the Checkmate 141 trial showed that 58.9% of patients treated with nivolumab experienced adverse reactions, among which 13.1% had grade 3 to 4 adverse reactions. Several HNSCC patients (62%–64%) receiving pembrolizumab experienced adverse reactions, with 9%–17% of them falling in grade 3–4. In order to reduce the resistance to standard treatments, optimize treatment efficacy, and monitor adverse reactions, several ICI clinical combination treatments are being developed (Table 1).

HNSCC mutation

Summary of gene mutation results

The HNSCC patient gene mutation data were downloaded from the TCGA database based on four processing software. The "maftools" package in the R software was used to draw waterfall diagrams of the mutation results processed by the mutect. The top 30 genes with higher mutation probability were enriched in the waterfall chart, with the mutation types and probabilities of related genes in each sample. Different mutation types are represented by different colors, including frame-del mutations, nonsense mutations, missense mutations, frame shift ins, splice sites, frame shift del, start site mutations (translation start site), and multiple mutations coexist (multiple hits). The top three genes with the highest mutation probabilities were *TP53*, *TTN*, and *FAT1*, with mutation probabilities of 66%, 35%, and 21%, respectively (Fig. 1).

Analysis of gene mutation

Fig. 2a showed a total of nine common types of mutations in HNSCC samples, which is different from

Table 1 Ongoing clinical trials of Immune checkpoint inhibitors on HNSCC

Agent	Immune checkpoint	Combination	Phase	Clinical trials / NCT number	No.of patients	Predict time of completion
Relatlimab	LAG-3	Nivolumab	Phase I /II	NCT01968109	1500	December 31, 2023
Nivolumab	PD-1	Relatlimab	Phase II/III	NCT03470922	700	March 16, 2022
INCAGN02390	TIM-3	/	Phase I	NCT03652077	41	January 31, 2021
Pembrolizumab	PD-1	AST-008	Phase II	NCT03684785	130	September 30, 2021
AMP-110	B7-H4	/	Phase I	NCT01878123	26	July, 2014
Nivolumab	PD-1	Surgery, Radiotherapy/Chemoradiotherapy	Phase II	NCT03721757	120	November 2023
Nivolumab	PD-1	Ipilimumab	Phase II	NCT03406247	140	February 2024
Nivolumab	PD-1	Ipilimumab	Phase III	NCT02741570	947	February 4, 2026
Nivolumab	PD-1	Ipilimumab	Phase II	NCT02823574	675	January, 2024
Ipilimumab/ Nivolumab	PD-1/CTL-4	INCAGN01876	Phase I /II	NCT03126110	45	October, 2021

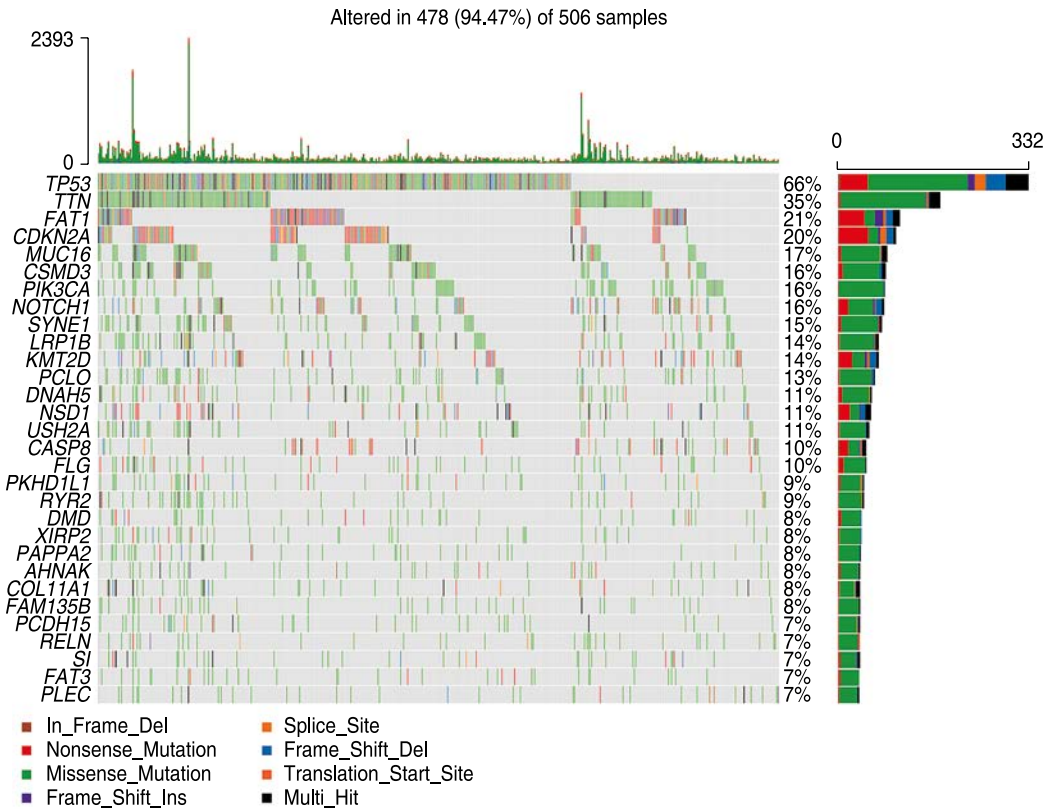

Fig. 1 Waterfall plot of tumor mutation

the analytical results of specific gene mutations in Fig. 1. It provides an overview of all high-probability mutation types in HNSCC patients, including missense mutations, nonsense mutations, frame shift del, frame shift ins, frame del, frame ins, splice sites, nonstop mutations, and translation start sites, of which missense mutations have the highest proportion. In addition, mutations at single nucleotide sites occurred more frequently than insertions or deletions (Fig. 2b). Among them, C > T mutations are the most commonly found single-nucleotide variant (SNV) types in HNSCC (Fig. 2c). Fig. 2d and 2e further summarize the total number of mutations with categories for each sample. The top 10 genes with the highest mutation probabilities in HNSCC samples were identified as *TP53* (66%), *TTN* (35%), *FAT1* (21%), *MUC16* (17%), *CDKN2A* (20%), *CSMD3* (16 %), *SYNE1* (15%), *LRP1B* (14%), *NOTCH1* (16%), and *PIK3CA* (16%) (Fig. 2f). According to previous research, HNSCC is a heterogeneous tumor and is related to classic pathogenic factors such as smoking and drinking [13]. Therefore, the appearance of tobacco-related genes such as TP53 and *CDKN2A* with higher mutation frequency in HNSCC patients in this habit and behavior independent prediction validated the design of the study.

Correlation analysis of mutant genes

The correlation analysis between genes with higher mutation probability revealed mutually exclusive relationships as the most common predictive correlation, while the co-expression relationship was more significant (Fig. 3). Among them, the green color represents the co-expression relationship of the two genes, and the red color represents the mutual exclusion relationship; the significance of the correlation was non-significant, significant ($P < 0.05$), and highly significant ($P < 0.001$). Among them, there is a highly significant positive correlation between *TP53* and *CDKN2A* genes ($P < 0.001$), which highlights the potential for further exploration as a possible key theoretical research direction.

HNSCC mutant genes and ICI therapy

Following the discoveries above, the mechanism between higher mutation frequency genes and ICI therapy in HNSCC was further explored and analyzed.

Higher mutation frequency genes

TP53 gene

The latest research results in 2021 show that the immune-related gene prognostic index (IRGPI) can be

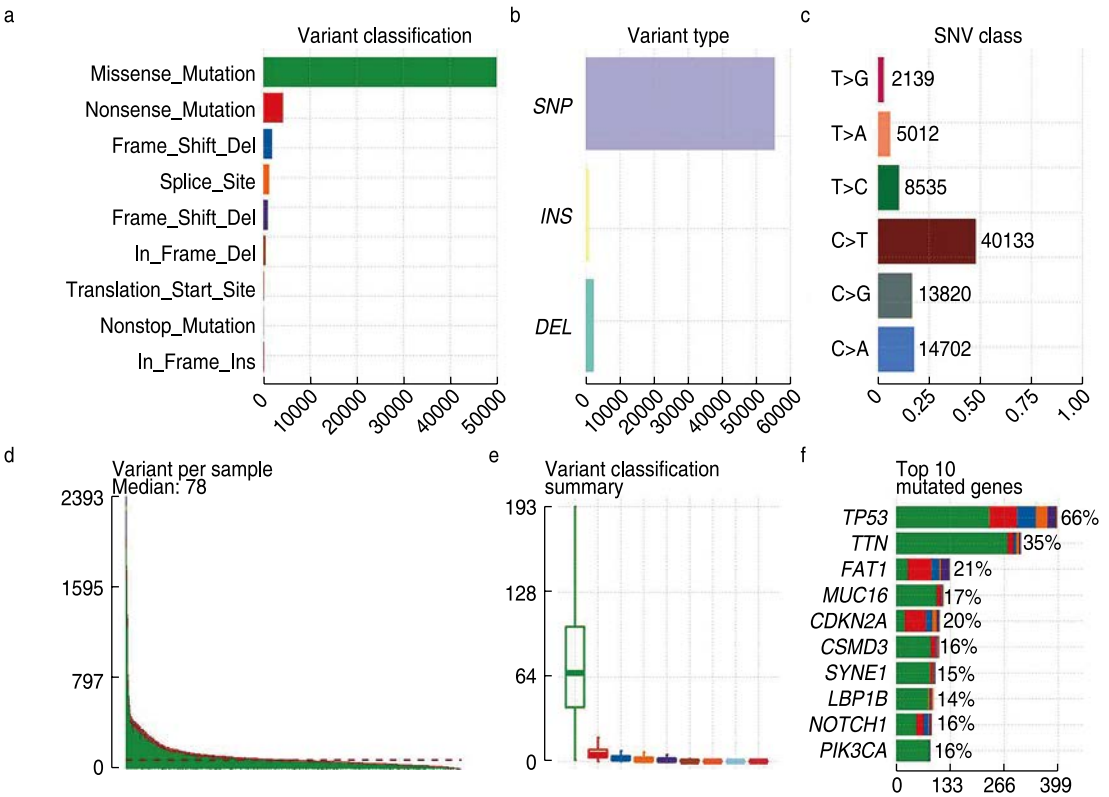

Fig. 2 Summary of mutation type. (a) Among the variant classification category, missense mutation accounted for the majority; (b) SNP is the most frenquecy variant type in HNSCC; (c) In SNV class, C > T account for 40133 cases and is the most commom SNV type in HNSCC; (d) The total mutation number in each sample; (e) Box plots of each variant classification in each sample; (f) Top 10 mutated genes in HNSCC with the variant frenquency. SNP, single nucleotide polymorphism; SNV, single nucleotide variants

used as a predictive marker of post-ICI treatment efficacy in HNSCC patients. A higher IRGPI indicates better treatment outcomes of the patients; in contrast, low IRGPI indicates poor ICI treatment effect. In this study, it was seen that there was a significant negative correlation between the mutation frequency of the *TP53* gene in HNSCC patients with IRGPI expression, further indicating that the former can predict the ICI treatment effect of HNSCC patients in the opposite way [14]. According to the literature, the underlying mechanism of this correlation may be as follows: HPV is one of the triggers of HNSCC. The viral genome integration into the host cell genome causes E6 and E7 to express viral oncoproteins, which leads to the degradation of TP53, inactivation of tumor suppressor retinoblastoma protein, and activation of the immunosuppressive pathway to allow tumor escape [13, 15–16]. Therefore, the occurrence of *TP53* gene mutations can affect their interaction with viral oncoproteins. The efficacy of ICI therapy changes accordingly by regulating the degradation efficiency of TP53 [17].

PI3KCA gene

The IRGPI article reported that there is a significant correlation between the low mutation frequency of *PI3KCA* and *IRGPI* in HNSCC patients, while the high-frequency mutation group had no such correlation. It also predicted that the prognostic results are similar to the *TP53* mutation frequency, which suggests that HNSCC patients in the low mutation frequency group of *PI3KCA* tend to obtain better ICI treatment effects. In addition, compared with the wild-type with HNSCC in a mouse model, the *PI3K* knockout can regulate T cells and immune checkpoint markers (PD-L1, PD-1) by affecting the functions of myeloid cells and T cell expression, thereby increasing the expression of anti-tumor cytotoxic molecules (IFN-γ, IL-17). These results indicate that the inhibition of *PI3K* can regulate the expression of tumor-related immune cells, indicating that the use of *PI3K* inhibitors in combination with ICI can further enhance the therapeutic effect of HNSCC [18]. In 2020, Novartis invented the world's first *PIK3CA* mutation medicine, Piqray, which was approved for the Canadian market, targeting advanced breast cancer. The phase III clinical trial results reveal significantly prolonged mPFS of patients (11.0 months vs 5.7 months) who underwent combined treatment of Piqray and fulvestrant, while the ORR increased nearly 2 times (36% vs. 16%) [19], proving that targeting *PI3KCA* mutations is significantly promising for tumor treatment. Although there have

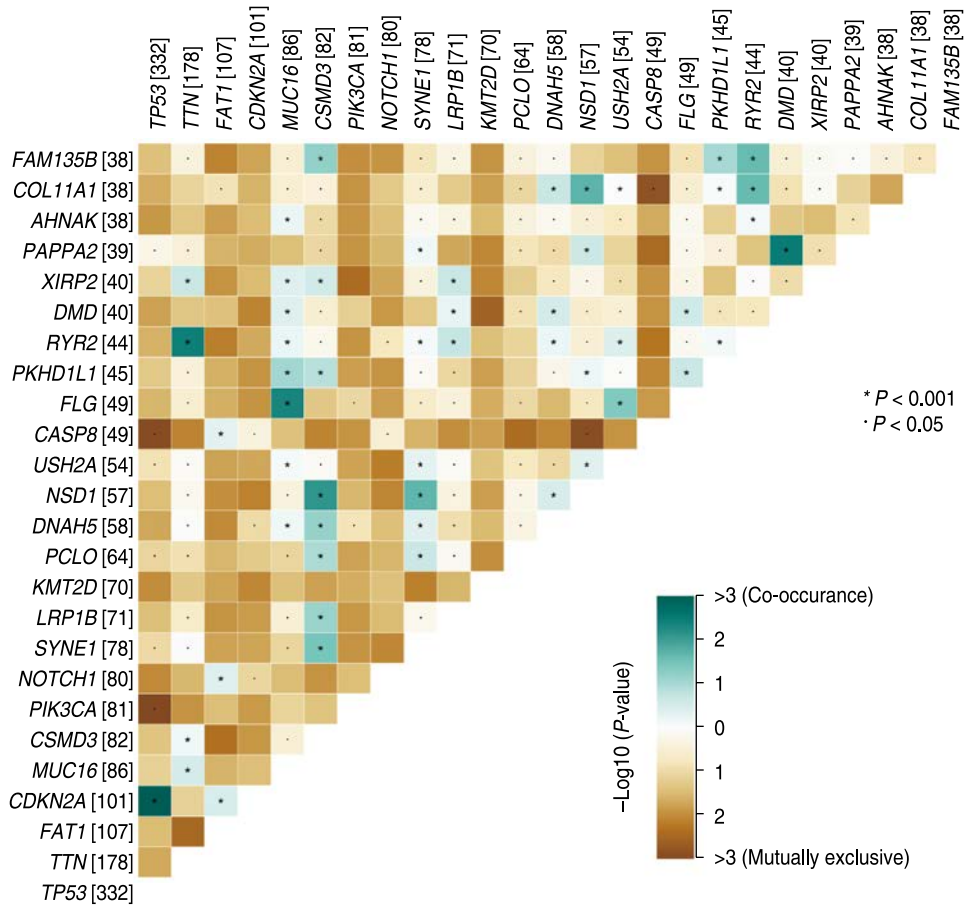

Fig. 3 Correlation analysis of mutated genes

been few studies on the correlation between post-ICI HNSCC and *PI3KCA* mutation types, we assume that by analyzing the type and frequency of *PI3K* mutations in HNSCC samples, the therapeutic effect of *PI3K* inhibitors can be further determined to identify novel strategies for ICI treatment combination.

NOTCH1 gene

The results of 126 HNSCC patients who underwent ICI treatment suggested that the frequency of *NOTCH1* mutation is related to the immune response to PD-1/L1 inhibitors, and the high frequency of *NOTCH1* mutation was more likely to occur in HPV-negative anti-PD-1/PD-L1 responders ($P < 0.05$) [20]. A recent study showed that *NOTCH1* mutation can be used as a new biomarker for lung cancer patients receiving ICI treatment, which consistently refers to the *NOTCH1* mutation as an important predictor of ICI treatment effect; however, the relevant mechanism remains unclear [21].

Signal pathways related to mutant genes

Wnt signaling pathway

Considering the highly frequent mutant genes (e.g., *FAT1* and *NOTCH1*), the results in Fig. 3 show a significant positive correlation between these two genes. The reason for this co-expression correlation could be that both *FAT1* and *NOTCH1* genes exist in the abnormally activated Wnt signaling pathway, which is involved in the development of tumors. Studies have shown that the Wnt signaling pathway can cooperate or antagonize other signaling pathways to further regulate tumor proliferation, migration, and invasion. Additionally, its constitutive expression can eliminate T cells in tumor tissues and contribute to resistance to ICI treatment [22]. An HNSCC study demonstrated that *FAT1* and *NOTCH1* are upstream conditional factors of the Wnt signaling pathway. Mutations in these two genes can lead to the loss of the core component of pathway-β-catenin, inhibiting the cancer process [17]. Therefore, an in-depth study of *FAT1* and *NOTCH1* mutations in the Wnt pathway can help further the understanding of the mechanism of drug resistance in HNSCC patients.

Hippo-YAP signaling pathway

The *PI3KCA* gene is located in the PI3K/Akt/mTOR signaling pathway, a classic pathway of immune

resistance after ICI treatment. There have been studies that have found that the *PI3KCA* gene is highly likely to be closely related to another tumor immune pathway, namely the Hippo-YAP signaling pathway. The high expression of *PI3KCA* in HNSCC patients is related to nuclear YAP localization, which can activate downstream target genes to promote the growth of HNSCC tumor cells, causing HNSCC patients to have a higher tumor recurrence rate [23]. In contrast, the latest research in 2021 shows that YAP expression is negatively correlated with the prognosis of patients with solid tumors, which can mediate the resistance to anti-PD-1 treatments and become a biological predictor of the efficacy of anti-PD-1 treatments [24]. Consequently, we speculate that the high frequency of *PI3KCA* mutations in HNSCC patients will affect the expression of YAP, thereby further affecting the tumor recurrence rate and patients' ICI treatment effect.

Discussion

This is the first comprehensive discussion on the analysis of HNSCC mutation results based on the TCGA database that analyzes the effects of ICI treatment and related immune mechanisms in patients. To date, ICIs still have limitations in the application of HNSCC. Although ICI has made breakthroughs in other solid tumor treatment options, the clinical data for its application are still insufficient. Studies have shown that the mechanism of PD-L1 expression in HNSCC and other solid tumors may be partially different, and not all HNSCC patients are PD-L1 positive; hence, more data are required to further guide clinical applications. On the other hand, drug tolerance leads to a decline in long-term therapeutic effects, which triggers the alteration towards a combination of multiple immune checkpoint inhibitors currently under development. Theoretically, a customized treatment plan according to the patient's marker expression or gene mutation case can improve the curative effect of HNSCC more precisely while reducing side effects and other adverse reactions. Nevertheless, the present supportive technology system for precision medical treatment setup in our country, such as the establishment of biobanks, bioinformatics collection and analysis, and big data analysis technology, are not yet mature. Therefore, this article collected the gene mutation information of HNSCC patients in public databases, conducted preliminary analysis through bioinformatics, and elaborated the relevant mechanisms, aiming to provide better treatment plans for HNSCC patients under the guidance of precise treatment plans in the future.

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Conflicts of interest
The authors indicated no potential conflicts of interest.

Author contributions
Hua Yang and Yuxue Wei contributed to data acquisition and data analysis, and Gangli Liu took responsibility for the data interpretation and reviewed and approved the final version of this manuscript.

Data availability statement
Not applicable.

Ethical approval
Not applicable.

References

1. Chen SH, Hsiao SY, Chang KY, et al. New insights into oral squamous cell carcinoma: from clinical aspects to molecular tumorigenesis. Int J Mol Sci. 2021;22(5):2252.
2. Singh S, Hassan D, Aldawsari HM, et al. Immune checkpoint inhibitors: a promising anticancer therapy. Drug Discov Today. 2020;25(1):223-229.
3. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun. 2020;11(1):3801.
4. Lee TW, Lai A, Harms JK, et al. Patient-derived xenograft and organoid models for precision medicine targeting of the tumour microenvironment in head and neck cancer. Cancers. 2020; 12(12):3743.
5. Nishikawa D, Sasaki E, Suzuki H, et al. Treatment outcome and pattern of recurrence of sinonasal squamous cell carcinoma with EGFR-mutation and human papillomavirus. J Craniomaxillofac Surg. 2021;49(6):494-500.
6. Chahoud J, Gleber-Netto FO, McCormick BZ, et al. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma. Clin Cancer Res. 2021;27(9):2560-2570.
7. Zandberg DP, Strome SE. The role of the PD-L1:PD-1 pathway in squamous cell carcinoma of the head and neck. Oral Oncol. 2014; 50(7):627-32.
8. Majc B, Novak M, Jerala NK, et al. Immunotherapy of glioblastoma: current strategies and challenges in tumor model development. Cells. 2021;10(2):265.
9. Ferris RL, Blumenschein Jr G, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med. 2018;81:45-51.
10. Seiwert TY, Burtness B, Mehra R, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol. 2016;17(7):956-965.

11. Bauml J, Seiwert TY, Pfister DG, et al. Pembrolizumab for platinum- and cetuximab-refractory head and neck cancer: results from a single-arm, phase II study. J Clin Oncol. 2017;35(14):1542-1549.
12. Cohen E, Harrington K, Le Tourneau C, et al. Pembrolizumab (pembro) vs standard of care (SOC) for recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC): phase 3 KEYNOTE-040 trial. Ann Oncol. 2017;28:628.
13. Solomon B, Young RJ, Rischin D. Head and neck squamous cell carcinoma: Genomics and emerging biomarkers for immunomodulatory cancer treatments. Semin Cancer Biol. 2018;52(Pt 2):228-240.
14. Chen Y, Li ZY, Zhou GQ, et al. An immune-related gene prognostic index for head and neck squamous cell carcinoma. Clin Cancer Res. 2021;27(1):330-341.
15. Biton J, Mansuet-Lupo A, Pécuchet N, et al. TP53, STK11, and EGFR mutations predict tumor immune profile and the response to anti–PD-1 in lung adenocarcinoma. Clin Cancer Res. 2018;24(22):5710-5723.
16. Offin M, Chan JM, Tenet M, et al. Concurrent RB1 and TP53 alterations define a subset of EGFR-mutant lung cancers at risk for histologic transformation and inferior clinical outcomes. J Thorac Oncol. 2019;14(10):1784-1793.
17. Leemans CR, Snijders PJ, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018;18(5):269-282.
18. Anderson K, Ryan N, Alkhimovitch A, et al. Inhibition of PI3K isoform p110γ increases both anti-tumor and immunosuppressive responses to aggressive murine head and neck squamous cell carcinoma with low immunogenicity. Cancers. 2021;13(5):953.
19. Rugo H, André F, Yamashita T, et al. Time course and management of key adverse events during the randomized phase III SOLAR-1 study of PI3K inhibitor alpelisib plus fulvestrant in patients with HR-positive advanced breast cancer. Ann Oncol. 2020;31(8):1001-1010.
20. Hanna GJ, Lizotte P, Cavanaugh M, et al. Frameshift events predict anti-PD-1/L1 response in head and neck cancer. JCI Insight. 2018;3(4):e98811.
21. Zhang K, Hong X, Song Z, et al. Identification of deleterious NOTCH mutation as novel predictor to efficacious immunotherapy in NSCLC. Clin Cancer Res. 2020;26(14):3649-3661.
22. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature. 2015;523(7559):231-235.
23. García-Escudero R, Segrelles C, Dueñas M, et al. Overexpression of PIK3CA in head and neck squamous cell carcinoma is associated with poor outcome and activation of the YAP pathway. Oral Oncol. 2018;79:55-63.
24. Yu M, Peng Z, Qin M, et al. Interferon-γ induces tumor resistance to anti-PD-1 immunotherapy by promoting YAP phase separation. Mol Cell. 2021;81(6):1216-1230.

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