

CD3D: a prognostic biomarker associated with immune infiltration and immunotherapeutic response in head and neck squamous cell carcinoma

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ABSTRACT

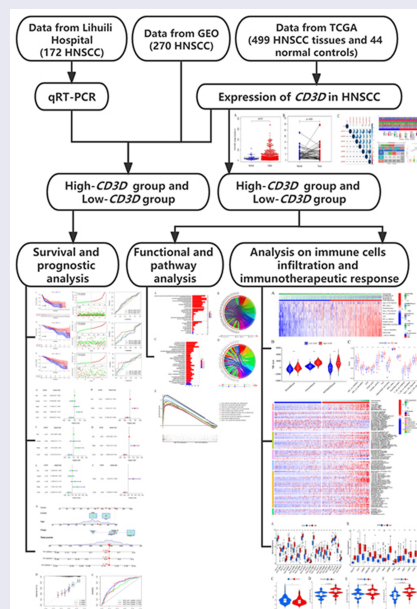
Recent studies have demonstrated that *CD3D* activates T-cell-related signal transduction and is associated with the antitumor immune response in several cancers. This study explored the role of *CD3D* in head and neck squamous cell carcinoma (HNSCC). A total of 499 HNSCC tissues and 44 normal controls were acquired from The Cancer Genome Atlas as the training cohort. GSE65858 included 270 HNSCC patients and was obtained from the Gene Expression Omnibus database as the test cohort. Overall, 172 HNSCC patients were collected as the validation cohort. *CD3D* expression in the validation cohort was measured by quantitative real-time polymerase chain reaction. The Kaplan–Meier plot revealed that high *CD3D* expression was associated with longer overall survival in HNSCC patients. Univariate and multivariate analyses showed that *CD3D* expression was an independent prognostic factor for HNSCC patients, which was confirmed in the test cohort and validation cohort. Furthermore, GO, KEGG, and GSEA analyses revealed the association of *CD3D* with immune-related pathways. Subsequently, ESTIMATE analysis showed the association between *CD3D* and the tumor microenvironment, while ssGSEA showed a remarkable positive link between *CD3D* and immune-related functions. Multiple algorithms demonstrated that high *CD3D* expression was associated with more immune effector cell infiltration. Finally, the tumor immune dysfunction and exclusion (TIDE) score and immunophenoscore (IPS) showed that patients with high *CD3D* could benefit from immunotherapy. In summary, *CD3D* was an independent favorable prognostic biomarker and correlated with immune cell infiltration and immune-related function, as well as an efficient indicator of immunotherapy response for HNSCC patients.



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Highlights

- *CD3D* was an independent prognostic factor of HNSCC cases.
- *CD3D* was associated with immune related signaling and immune cells infiltration, as well as involved in immune related function in HNSCC patients.
- HNSCC patients with high *CD3D* expression might respond better to immunotherapy.

Introduction

Head and neck cancer is the sixth most prevalent malignancy worldwide. In addition, head and neck cancer is a major cause of cancer-associated mortality worldwide, ranking fifth [1]. The International Agency for Research on Cancer reported that more than 878,000 new head and neck malignancy cases were diagnosed worldwide in 2020, with approximately 444,000 deaths [2]. Greater than ninety percent of head and neck cancers are head and neck squamous cell carcinoma (HNSCC) originating from the hypopharynx, oropharynx, lip, nasopharynx, oral cavity, and larynx [3]. The predominant risk factors are heavy consumption of tobacco and alcohol and human papillomavirus infection [4]. Current treatment for HNSCC relies on combined therapy mainly based on surgery. Recently, immune checkpoint inhibitors (ICIs), which include anti-programmed cell death 1 (PD-1) and anti-programmed cell death ligand 1 (PD-L1) treatments, have also been used in the management of HNSCC [5]. However, local recurrence and distal metastasis remain major challenges [6], and the 5-year survival rate of patients with HNSCC is still <50% [7]. Therefore, new molecular markers and therapeutic interventions should be discovered to enhance patient prognosis and provide more accurate survival prediction.

T-lymphocytes are vital players in the antitumor immunological response. They contribute to triggering and controlling the adaptive immune response and activating cytotoxic feedback in tumors [8]. Ligation of the T-cell antigen

receptor is critical for adaptive immune responses to occur. *CD3D* is one of the components of the T-cell receptor (TCR)/CD3 complex and functions in the signal transduction of T-cell activation. A TCR-mediated signal is conducted across the plasmalemma through the CD3 chain upon extracellular binding of the TCR to antigen-presenting cells. All CD3 chains, which include *CD3G*, *CD3D*, *CD3E*, and *CD3Z*, possess immunoreceptor tyrosine-based stimulation motifs in their cytoplasmic structural domains. These motifs undergo phosphorylation when acted upon by the Src family protein tyrosine kinases LCK and FYN, resulting in the activation of downstream signaling pathways [9]. Furthermore, the CD3 chains play important roles in several cancers [10–13], including HNSCC. High expression of *CD3E* [14,15] and *CD3G* [15,16] was significantly associated with a good prognosis in HNSCC patients, and *CD3Z* was related to pretreatment pain in patients with HNSCC [17]. However, the function of *CD3D* in HNSCC is still unknown. *CD3D* is one of the components of the TCR/CD3 complex and functions in the signal transduction of T-cell activation [18]. In addition, *CD3D* functions in thymocyte differentiation. Thymocytes fail to differentiate appropriately without a functioning TCR/CD3 complex [19]. Moreover, previous studies reported that *CD3D* could act as a biomarker for cancers. Yang et al. suggested that *CD3D* could function as a prognostic marker for colon cancer and influence the development of immunotherapy [20]. According to Zhu et al., *CD3D* is a predictive biomarker of the prognosis of breast carcinoma associated with lymphocyte infiltration and immune checkpoints [21].

In the current research, we aimed to explore the prognostic value of *CD3D* expression for HNSCC patients utilizing data from TCGA database. The GEO database and 172 HNSCC subjects with survival information were available to confirm the findings. We also evaluated the association between *CD3D* expression and biological function, the tumor microenvironment (TME), infiltrating immune cells, and the response to immunotherapy in HNSCC.

Materials and methods

Sample acquisition

For this research, the *CD3D* RNA-seq data (FPKM form), methylation data, and corresponding clinicopathological data of 499 HNSCC tissues and 44 normal controls were retrieved from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>, last accessed: 17 July 2021) and used as the training cohort. The Gene Expression Omnibus (GEO) database was also retrieved to obtain the GSE65858 dataset (GPL10558 platform), which was utilized as the test cohort. The GSE65858 dataset records the transcriptome data of 270 HNSCC patients with survival data. In addition, 172 HNSCC patients treated surgically at Ningbo Medical Center Lihuili Hospital (Ningbo, China) between 2014 and 2018 were collected as the validation cohort. The human ethics committees of the Ningbo Medical Center Lihuili Hospital approved these experimental protocols (Approval Number: KY2022PJ049). Table 1 shows the detailed clinical information of the HNSCC patients in this study.

Quantitative reverse transcription-polymerase chain reaction and total RNA synthesis

The practical steps for total RNA synthesis and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) are outlined in our previous study [22]. The primer sequences for *CD3D* are shown below: 5'-ACTGGCTACCCTTCTCTCG-3' (forward primer) and 5'-CCGTTCCTCTACCCATGTGA-3' (reverse primer). *GAPDH* was simultaneously amplified as the internal control [23]. The primer sequences are shown in Table 2. The qRT-PCR parameters were as follows: the first was denaturation for 10 minutes at 95°C, followed by 40 cycles of 20 seconds at a temperature of 95°C, 60°C for 30 seconds, and 72°C for 30 seconds. All experiments were repeated three times, and the average threshold cycling (CT) value was recorded for analysis. The relative *CD3D* expression levels were computed by the $2^{-\Delta Ct}$ approach [24].

Survival and prognostic analysis

Utilizing the median value as the threshold value, we assigned patients into the high-*CD3D* and low-*CD3D* groups. The Kaplan–Meier survival plotter with log-rank test was used to explore the association between *CD3D* expression and overall survival for HNSCC patients. Univariate and multivariate Cox analyses were conducted to explore the prognostic value of *CD3D* for HNSCC patients. Notably, only variables with significant influences derived from the univariate statistics were included in the multivariate analysis. On this basis, a nomogram model was developed to anticipate the 1-, 3- and 5-year survival rates of HNSCC cases. Each factor corresponds to a score in the first row regarding the nomogram design. The total score is equal to the sum of all scores, and the straight line below it predicts the survival rates of HNSCC cases. Calibration curves were also created to evaluate the nomogram's predictive abilities. The prediction accuracy was assessed using receiver operating characteristic (ROC) curves. The closer the area under the ROC curve (AUC) is to 1, the better the prediction accuracy [25].

Functional and pathway analysis

The DESeq2 package ($|\log_2FC| > 1$, $P < 0.05$) was employed in this research to detect the differentially expressed genes (DEGs) between the high and low *CD3D* groups. Based on these DEGs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed utilizing the org.Hs.e.d package. Gene set enrichment analysis (GSEA, version 4.1.0) was utilized in functional enrichment analysis as previously described [22].

Analysis of immune cell infiltration and immunotherapeutic response

Tumor purity, ImmuneScore, StromalScore, and ESTIMATEScore were calculated for HNSCC samples using the ESTIMATE package. The sum of the ImmuneScore and the StromalScore is the ESTIMATEScore. A higher

Table 1. Clinical features of HNSCC individuals in the present research.

Variables	TCGA cohort		GEO cohort		Validation cohort		
	Number	Percent	Number	Percent	Number	Percent	
Gender							
	Female	133	26.65%	47	17.41%	38	22.09%
	Male	366	73.35%	223	82.59%	134	77.91%
Age							
	≤60	279	55.91%	130	48.15%	77	44.77%
	>60	220	44.09%	140	51.85%	95	55.23%
Histologic grade							
	G1-2	359	71.94%	-	-	117	68.02%
	G3-4	121	24.25%	-	-	55	31.98%
	Unknown	19	3.81%				
T stage							
	T1-2	177	35.47%	115	42.59%	106	61.63%
	T3-4	267	53.51%	155	57.41%	66	38.37%
	Unknown	55	11.02%				
N stage							
	N0	170	34.07%	94	34.81%	100	58.14%
	N1-3	236	47.29%	176	65.19%	72	41.86%
	Unknown	93	18.64%				
Clinical stage							
	Stage I-II	94	18.84%	55	20.37%	88	51.16%
	Stage III-IV	337	67.54%	215	79.63%	84	48.84%
	Unknown	68	13.63%				
Survival status							
	Dead	217	43.49%	94	34.81%	78	45.35%
	Alive	282	56.51%	176	65.19%	94	54.65%

ESTIMATEScore suggested the presence of abundant immune cells or stromal components in the tumor microenvironment. The immunoactivity of thirteen immune-related pathways was determined utilizing single-sample gene set enrichment analysis (ssGSEA) in the GSVA program [26]. The TIMER, EPIC, CIBERSORT, QUANTISEQ, ESTIMATE, MCPcounter, and XCELL algorithms were used to assess the immune cell infiltration between the low and high *CD3D* expression groups. The expression of HLA-related and ICI-related genes in the low and high *CD3D* expression groups was also compared. Moreover, the tumor immune dysfunction and exclusion (TIDE) score [27] and immunophenoscore (IPS) [28] were employed to determine the immunotherapeutic response for HNSCC

patients. A lower TIDE score and higher IPS suggest a better response to immunotherapy.

Statistical analysis

All statistical analyses were conducted utilizing R 4.1.0 software, which can also be applied for visualization. The correlation between *CD3D* expression and clinicopathological features was carried out utilizing the Wilcoxon test and chi-squared test. Spearman correlation analysis was applied to assess correlations. A two-tailed $P < 0.05$ was defined as significant.

Results

CD3D can act as a predictive biomarker in several cancers, but its role in HNSCC is not clear. In this study, *CD3D* expression was upregulated in HNSCC tissues and associated with longer overall survival in patients with HNSCC in the TCGA cohort. Univariate and multivariate analyses demonstrated that *CD3D* expression was an independent prognostic factor for HNSCC patients, which was verified by the GEO cohort and our validation cohort. Functional and

Table 2. List of primers used for the qRT-PCR assay.

Primer	Sequence (5' to 3')	Amplification size (bp)
<i>CD3D</i>		110
Forward	ACTGGCTACCCTTCTCTCG	
Reverse	CCGTTCCCTCTACCCATGTGA	
<i>GAPDH</i>		194
Forward	CCATGGAGAAGGCTGGGG	
Reverse	CAAAGTTGTCATGGATGACC	

pathway analyses revealed a strong association of *CD3D* with immune-related pathways. Further analysis showed that *CD3D* was associated with immune cell infiltration and was involved in immune function in HNSCC patients. HNSCC patients with high *CD3D* expression could benefit more from immunotherapy.

High expression of *CD3D* in patients with HNSCC

By analyzing the *CD3D* RNA-seq data from 499 HNSCC tissues and 44 normal controls in the TCGA cohort, we confirmed a remarkable upregulation of *CD3D* expression in HNSCC patients in contrast with normal tissue ($P < 0.01$, Figure 1(a)). However, there were no significant differences between the paired samples ($P = 0.891$, Figure 1(b)). Further analysis revealed a negative correlation between *CD3D* expression and the methylation level of CG sites at the *CD3D* promoter zone, especially for cg07728874, cg13750061, and cg24841244 (Figure 1(c)). Based on the TCGA cohort, we also probed the relationship between *CD3D* expression and the clinicopathological features of HNSCC cases. (Figure 2(a)). Both chi-squared ($P < 0.01$, Figure 2(b)) and Wilcoxon signed-rank (Figure 2(c)) tests demonstrated that the *CD3D* expression level significantly decreased in patients with advanced T stage compared with early T stage.

CD3D is an independent prognostic factor of HNSCC cases

To determine the relationship between *CD3D* expression and the prognosis of HNSCC cases, the patients in the three cohorts were divided into high and low *CD3D* expression groups on the basis of the median value. Kaplan–Meier curves and survival status distribution plots demonstrated that elevated *CD3D* expression levels were related to a substantially longer overall survival time in patients with HNSCC in the TCGA cohort (Figure 3(a,b), $P = 0.002$). The areas under the ROC curve (Figure 3(c)) for 1, 3, and 5 years were 0.574, 0.619, and 0.577, respectively. Additionally, the GEO

cohort (Figure 3(d,e), $P = 0.003$; figure 3(f), AUC of 1 year = 0.551, 3 years = 0.498, 5 years = 0.653) and the validation cohort (Figure 3(g,h), $P = 0.006$; Figure 3(i), AUC of 1 year = 0.585, 3 years = 0.612, 5 years = 0.653) showed consistent results with the TCGA cohort. Furthermore, univariate (Figure 4(a)) and multivariate Cox (Figure 4(b)) analyses demonstrated that *CD3D* expression was an independent predictive factor for the OS of HNSCC cases in the TCGA cohort (HR = 0.730, $P = 0.042$), which was confirmed in the GEO cohort (HR = 0.499, $P = 0.001$, Figure 4(c,d)) and validation cohort (HR = 0.536, $P = 0.008$, Figure 4(e,f)).

Construction of the nomogram based on *CD3D* expression

Construction of a predictive nomogram was performed to estimate the 1-, 3-, and 5-year survival rates of HNSCC individuals on the basis of age, sex, grade, T classification, N classification, stage, and *CD3D* expression (Figure 5(a)). The 1-, 3-, and 5-year calibration curves (Figure 5(b)) were all near the diagonal reference line, indicating good predictive performance. Figure 5(c) shows that the AUCs of the 1-, 3-, and 5-year ROC curves were 0.710, 0.761, and 0.757, respectively, indicating the nomogram's tolerable discrimination.

Functional enrichment analysis of *CD3D* in HNSCC

In total, 638 DEGs were detected between the two groups ($|\log_2FC| > 1$, $P < 0.05$). Then, GO and KEGG analyses based on these DEGs were conducted to determine the role played by *CD3D* in HNSCC. The GO analysis demonstrated that the DEGs were primarily enriched in immune-related functions, including immune responses, immunoglobulin complexes, and antigen binding (Figure 6(a,b)). KEGG analysis revealed that the DEGs primarily engaged in pathways such as Th1 and Th2 cell differentiation, cell adhesion molecules, and cytokine receptor interactions. Moreover, the

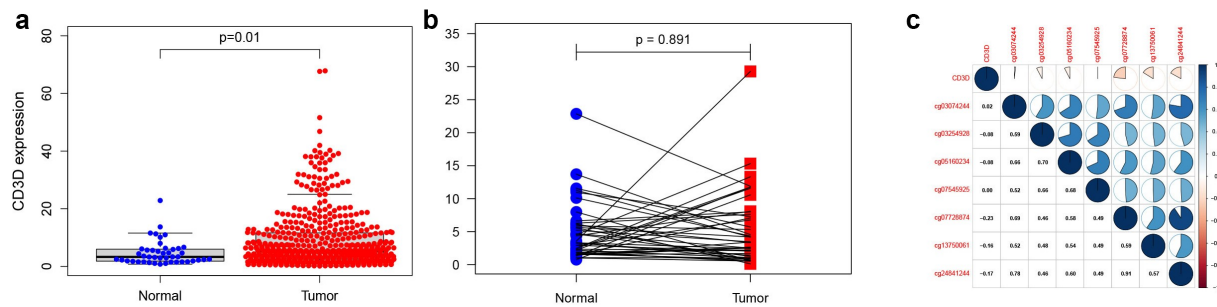


Figure 1. The expression and promoter methylation of *CD3D* in head and neck squamous cell carcinoma (HNSCC). (a) *CD3D* expression in 44 healthy controls and 499 HNSCC tissues from The Cancer Genome Atlas (TCGA) database. (b) *CD3D* expression in 44 paired HNSCC tissues and normal controls. (c) The association between the methylation levels of CG sites in the *CD3D* promoter and expression levels.

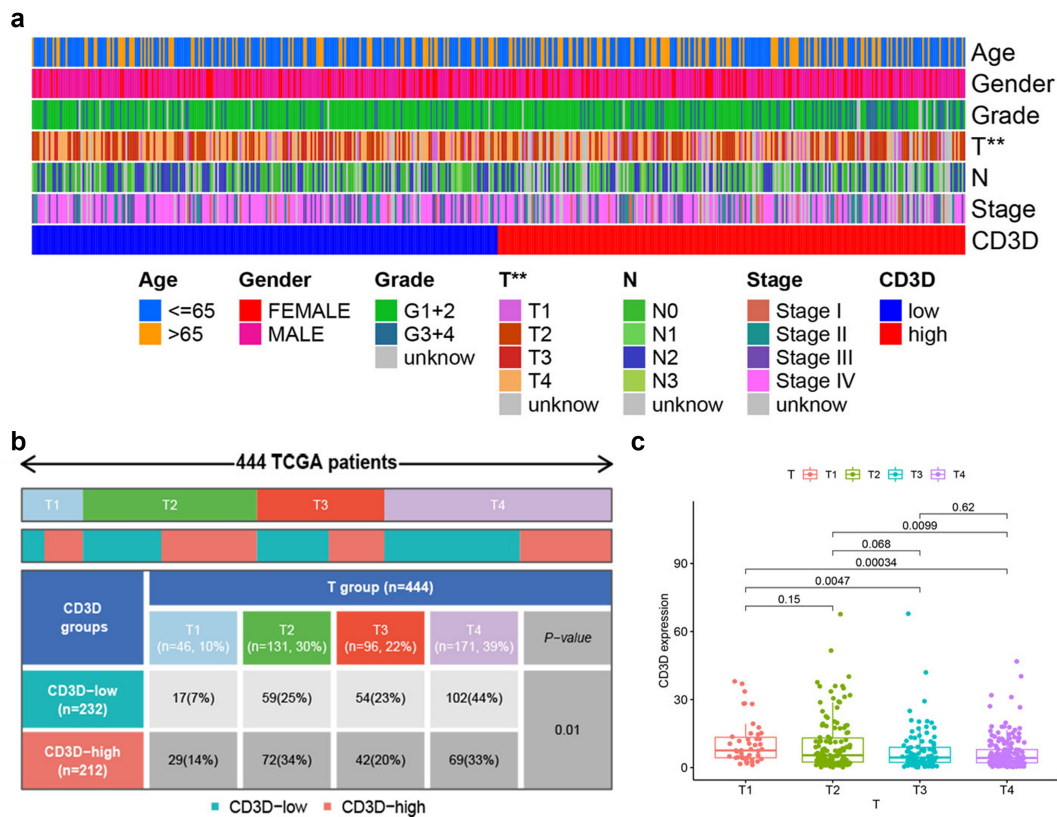


Figure 2. The link between the expression of *CD3D* and the clinicopathological characteristics among HNSCC individuals. (a) Heatmap demonstrating the distribution of the clinicopathological characteristics of HNSCC patients between the two groups. (b) With respect to the T stage between the two groups, a comparison was made by utilizing the chi-squared test. (c) The association between *CD3D* expression and T stage was analyzed using the Wilcoxon signed-rank test. ** $P < 0.01$.

function of *CD3D* in HNSCC was also investigated using GSEA. Ten KEGG pathways were substantially enriched in the high *CD3D* group (Figure 6(e)), including antigen processing and presentation, cell adhesion molecules, the B-cell receptor signaling pathway, cytokine–cytokine receptor interaction, the intestinal immune

network for IgA synthesis, the Jak-STAT signaling pathway, the cytosolic DNA sensing pathway, natural killer cell-mediated cytotoxicity, primary immunodeficiency, and the T-cell receptor signaling pathway. These significant signaling pathways were related to inflammatory and immune responses.

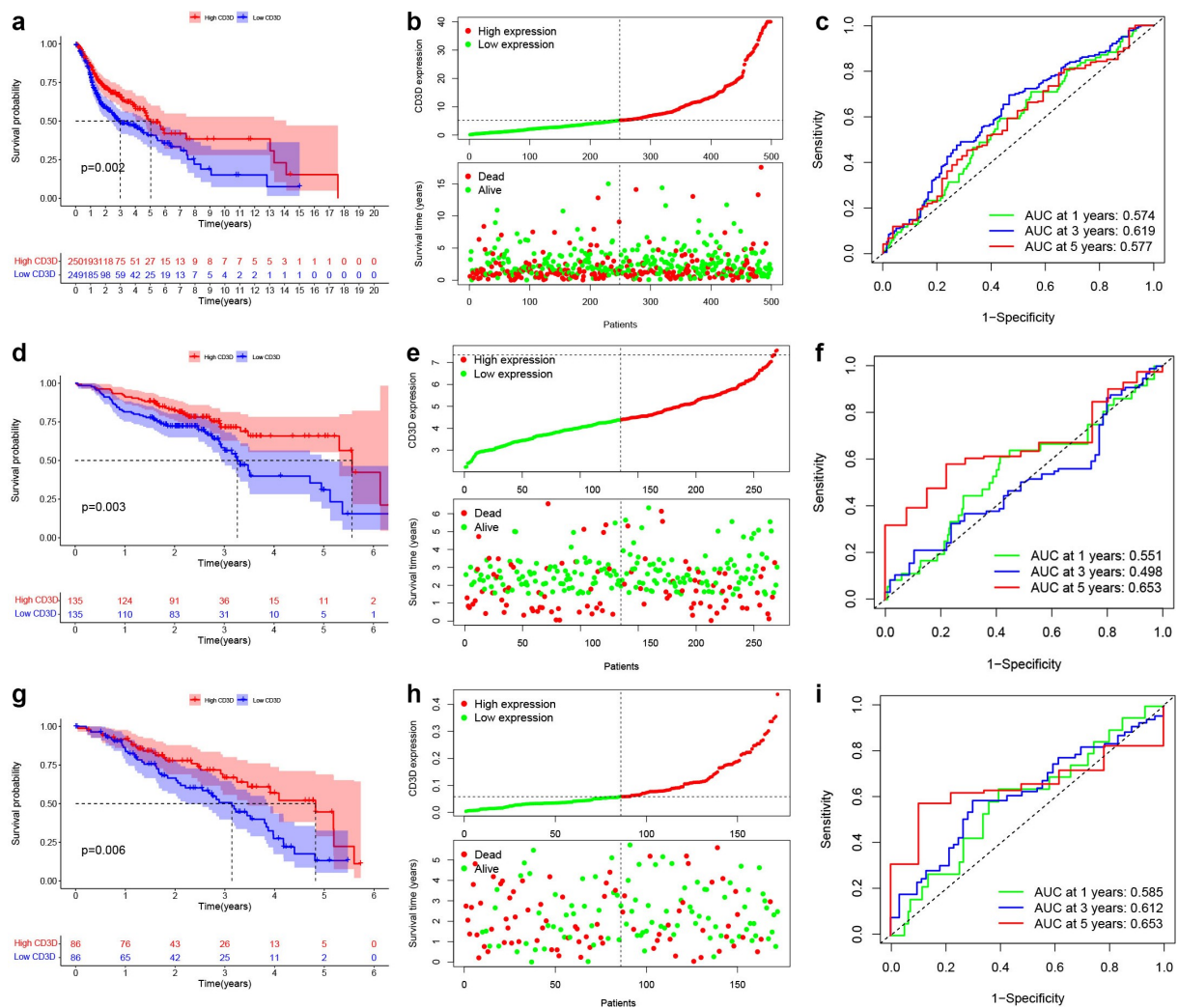


Figure 3. Expression of *CD3D* for anticipating overall survival in patients with HNSCC. (a) Survival curves of OS from the TCGA cohort ($n = 499$). (b) Distribution of *CD3D* expression, survival time, and survival status of the TCGA cohort. (c) ROC curves of the TCGA cohort. (d) Survival curves of OS from the GEO cohort ($n = 270$). (e) Distribution of *CD3D* expression, survival time, and survival status of the GEO cohort. (f) ROC curves of the GEO cohort. (g) Survival curves of OS from the validation cohort ($n = 172$). (h) Distribution of *CD3D* expression, survival time, and survival status of the validation cohort. (i) ROC curves of the validation cohort. TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; OS, overall survival.

Relationship between *CD3D* and immune cell infiltration in HNSCC

The heatmap illustrated differences in tumor purity, ImmuneScore, StromalScore, ESTIMATEScore, and ssGSEA score of immune-associated function between the two groups (Figure 7(a)). ESTIMATE analysis confirmed that the high *CD3D* expression group had a higher ImmuneScore, StromalScore, and ESTIMATEScore (Figure 7(b)) than the low *CD3D* expression group. Further ssGSEA demonstrated that the enrichment levels of immune-related functions increased in the high *CD3D* expression group

(Figure 7(c)), indicating that *CD3D* might be associated with immune cell infiltration and involved in immune function. As expected, multiple algorithms (Figure 8) demonstrated that the high *CD3D* expression group had more immune effector cell infiltration, including B cells, CD8 + T cells, and CD4 + T cells.

Relationship between *CD3D* expression and immunotherapeutic response in HNSCC

We analyzed HLA-related gene expression and ICI-related genes in the two groups. All 24

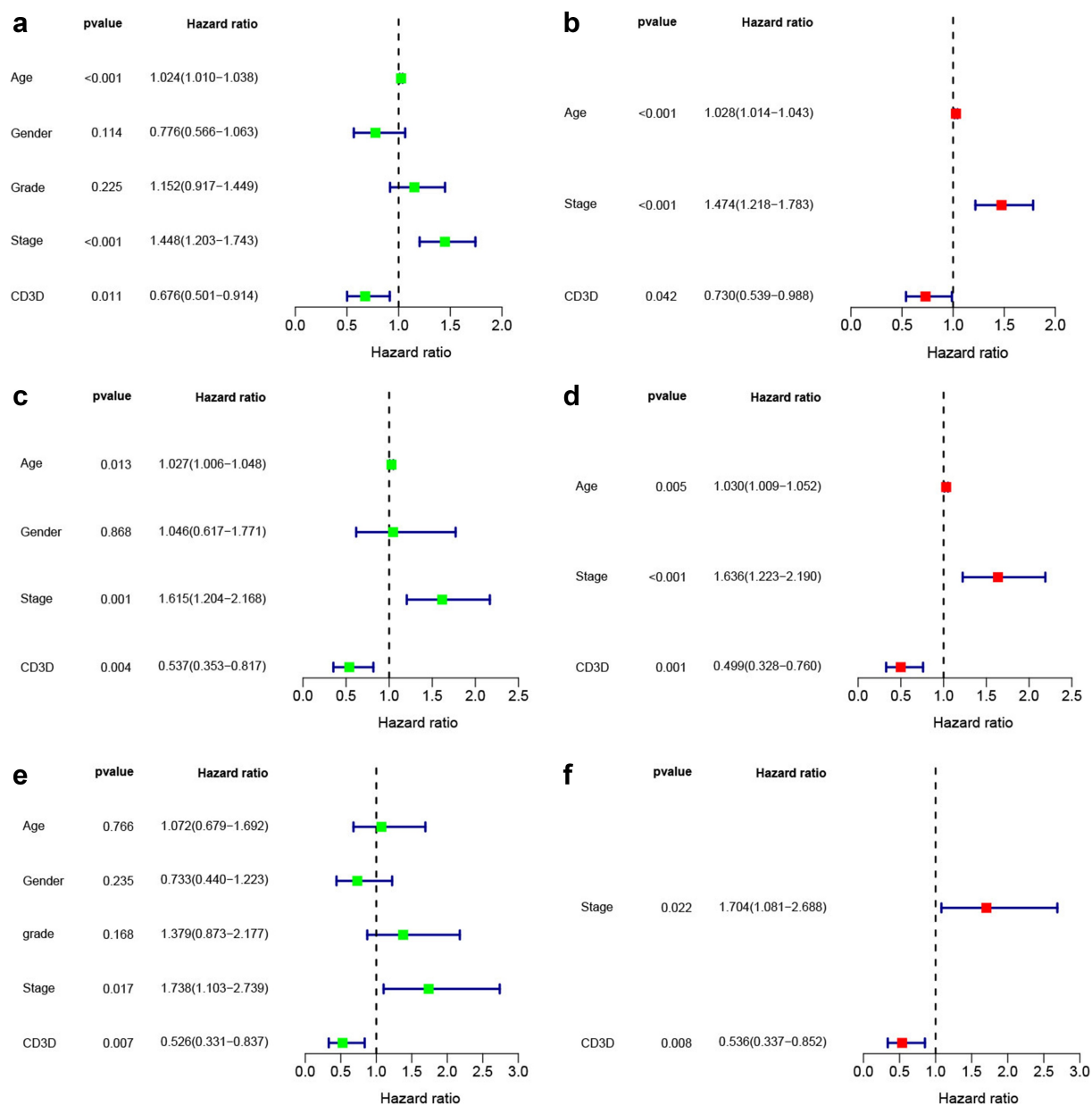


Figure 4. Univariate and multivariate Cox analyses. Univariate (a) and multivariate (b) Cox analyses of OS on the basis of *CD3D* expression after correcting for the clinicopathological characteristics in the TCGA cohort. Univariate (c) and multivariate (d) Cox analyses of OS according to the expression of *CD3D* after modifying the clinicopathological factors in the GEO cohort. Univariate (e) and multivariate (f) Cox analyses of OS according to the expression of *CD3D* after adjustment of the clinicopathological features in the validation cohort. TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; OS, overall survival.

HLA-related genes showed significantly higher expression levels in the high *CD3D* expression group than in the low *CD3D* expression group ($P < 0.001$, Figure 9(a)). Except for *TBX2*, we observed that 12 ICI-related genes were upregulated in the high *CD3D* expression group ($P < 0.05$, Figure 9(b)). In response to immunotherapy, cases in the high *CD3D* expression

group had reduced TIDE scores ($P < 0.001$, Figure 9(c)). HNSCC patients with high *CD3D* expression may respond better to immunotherapy. Additionally, whether anti-PD1 (Figure 9(d)) and anti-CTLA4 (Figure 9(e)) were used alone or in combination (Figure 9(F)), there were significantly increased IPS scores in the high *CD3D* expression group ($P < 0.001$).

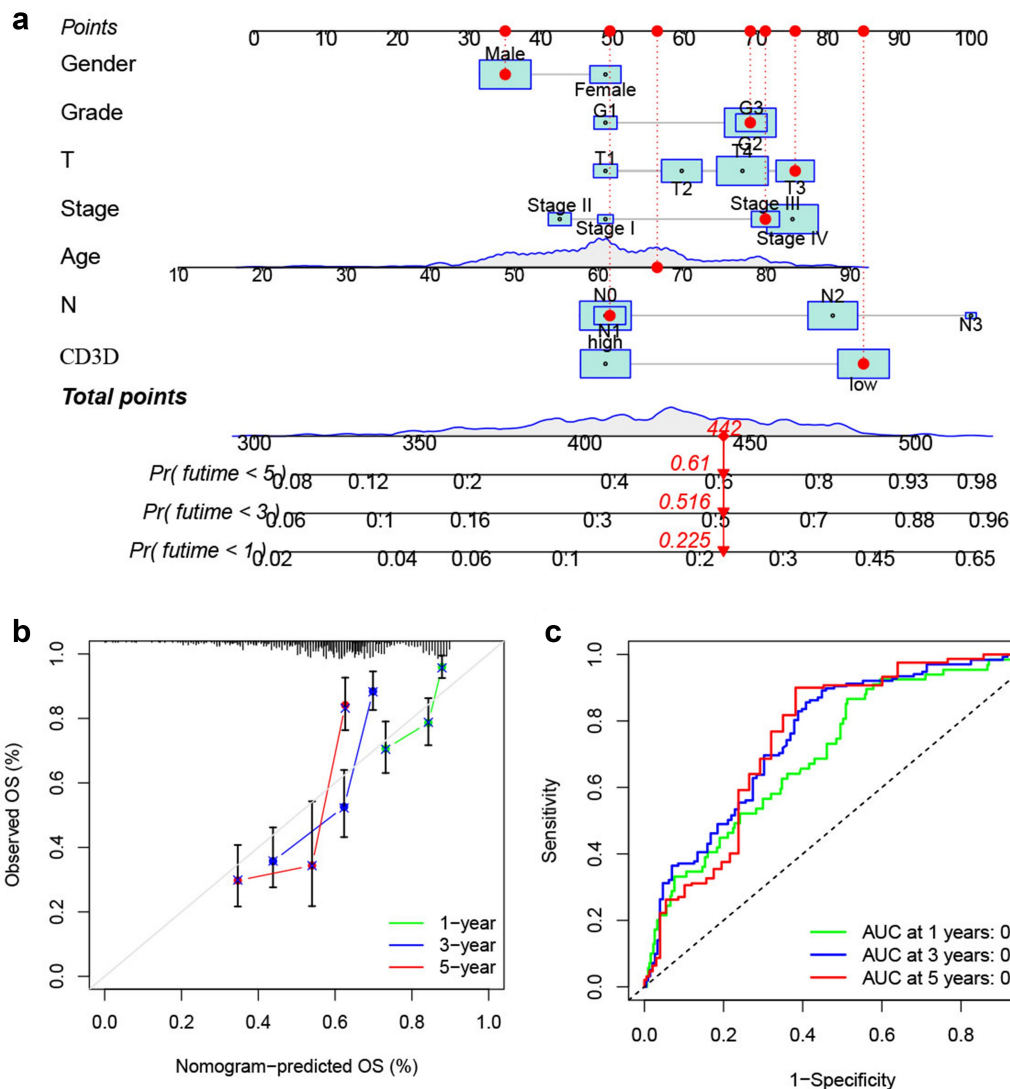


Figure 5. Construction and validation of a nomogram for HNSCC individuals. (a) Nomogram for prediction of the 1-, 3- or 5-year survival rates for HNSCC individuals. (b) Calibration curves of the nomogram. (c) Receiver operating characteristic curves of nomogram.

Discussion

T-lymphocytes play a crucial role in adaptive immune responses by recognizing antigens derived from pathogens or tumor cells. The activation of T-lymphocytes is tightly regulated by TCR signaling [29]. The multimeric TCR complex is composed of a clonotypic TCR $\alpha\beta$ or TCR $\gamma\delta$ heterodimer associated with invariant CD3 chains (CD3G, CD3D, CD3E, and CD3Z) [30]. Previous studies have investigated the effects of CD3E, CD3G, and CD3Z on HNSCC patients. As reported by Liu et al., the expression of CD3E in HNSCC tissues

was significantly higher than that in normal tissues adjacent to the tumor, which was a significant prognostic factor in recurrent HNSCC patients [14]. Wang et al. reported that high expression of CD3E and CD3G was significantly associated with a good prognosis in HNSCC patients [15]. In addition, Lecerf et al. showed that low CD3E expression was associated with a high risk of recurrence of HNSCC [16]. Moreover, Reyes-Gibby et al. found that CD3Z was important for pretreatment pain in HNSCC patients [17]. Nevertheless, it has been reported that CD3D

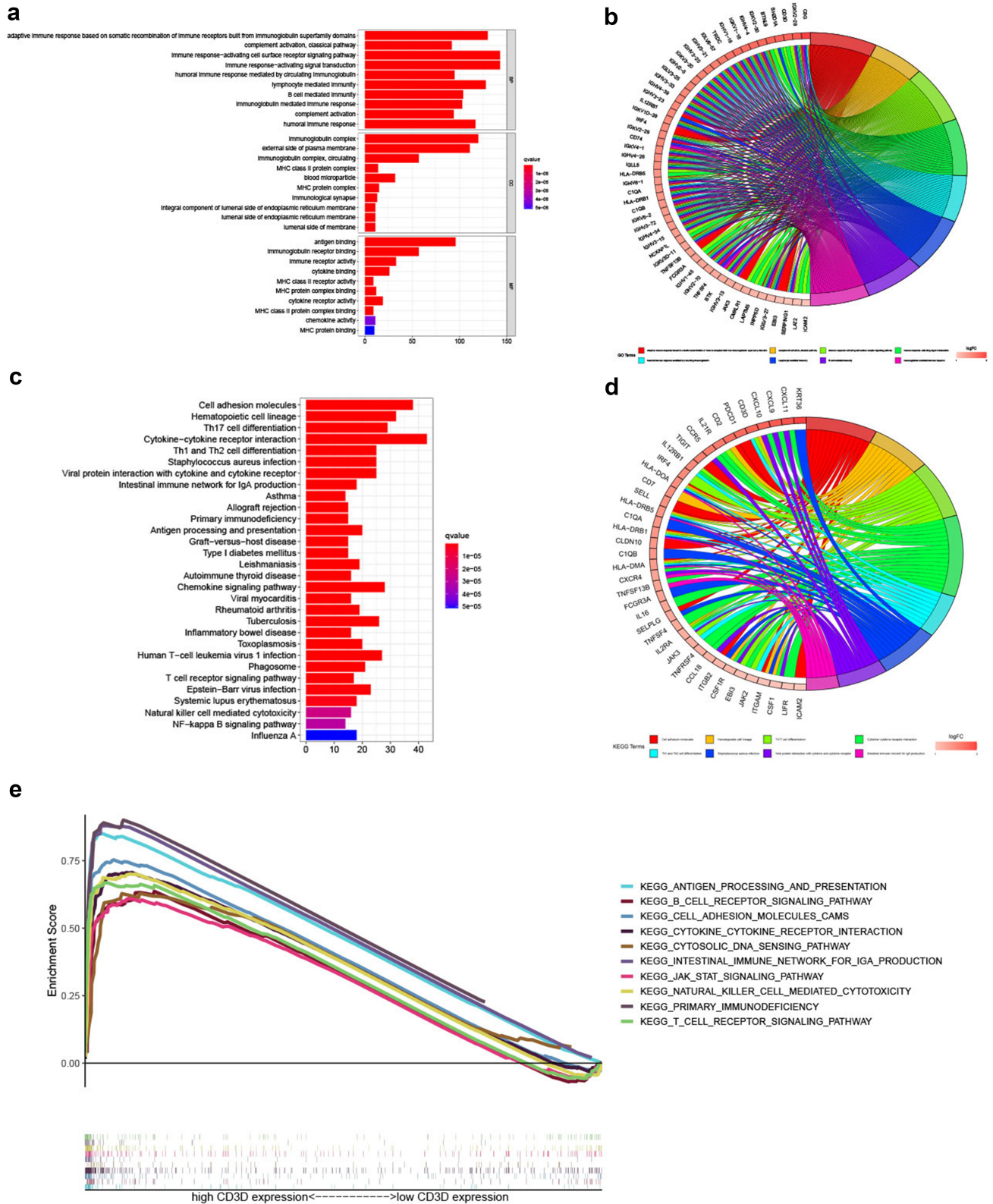


Figure 6. Functional enrichment analysis of *CD3D* in HNSCC. (a) Bar plot of the GO enrichment analyses. (b) Chord plot of the GO enrichment analyses. (c) Bar plot of the KEGG enrichment analysis. (d) Chord plot of the KEGG enrichment analysis. (e) GSEA between the two groups. GSEA, Gene Set Enrichment Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

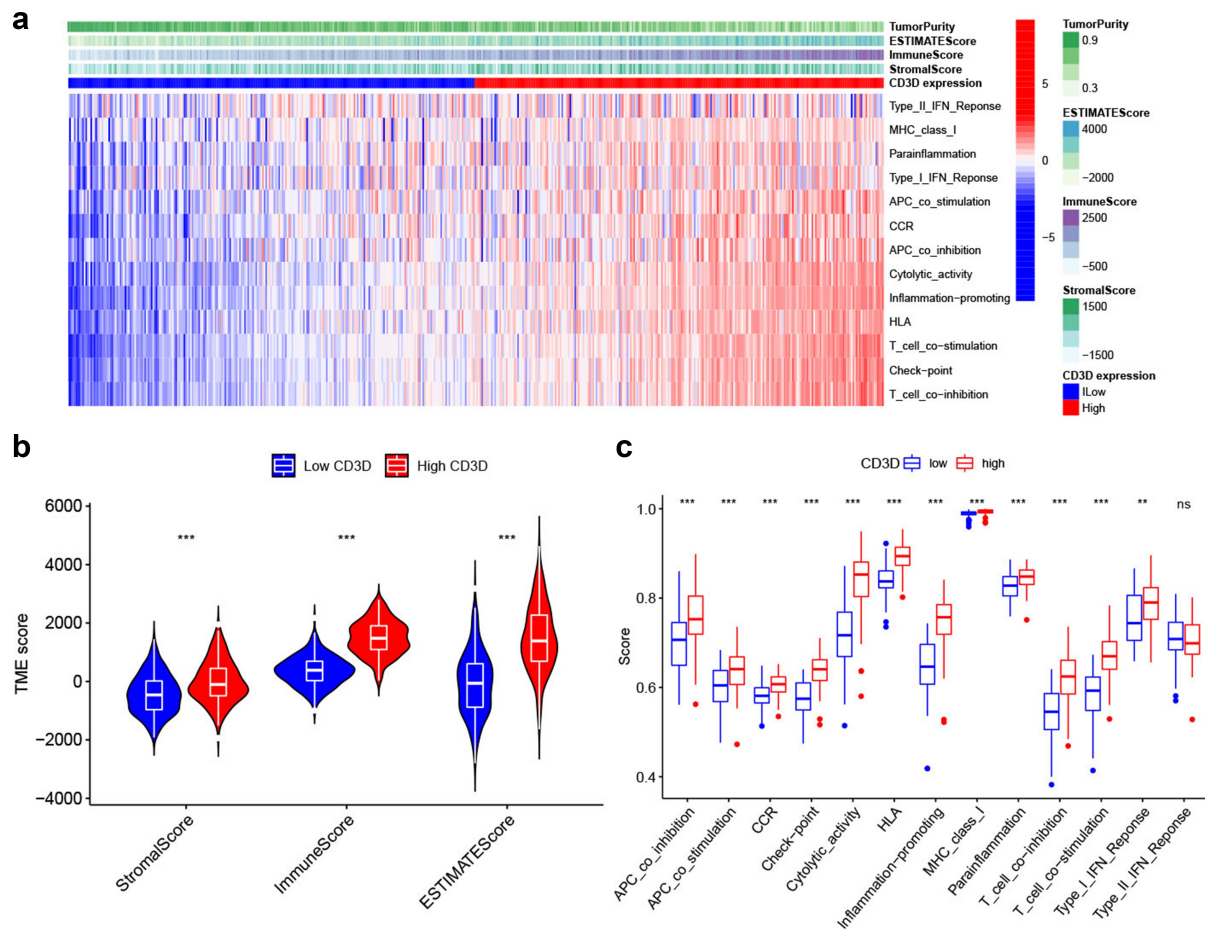


Figure 7. The effect of *CD3D* on the tumor microenvironment in HNSCC. (a) The heatmap shows the link between *CD3D* expression and the tumor immune microenvironment. (b) The stromal, immune, and ESTIMATE scores between the two groups utilizing the ESTIMATE algorithm. (c) Higher ssGSEA scores for most immune functions in the high *CD3D* expression group than in the low *CD3D* expression group. ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant.

could be used as a favorable prognostic biomarker for both colon cancer and breast cancer [20,21], and the function of *CD3D* in HNSCC has yet to be completely elucidated. This research demonstrated that *CD3D* was overexpressed in HNSCC tissues compared with normal samples based on the TCGA database. However, no significant differences were observed between the paired samples, which may have been due to an insufficient sample size. Furthermore, DNA methylation is an epigenetic alteration that plays an important role in gene expression regulation [31]. Bacolod et al. discovered a negative association between *CD3D* expression levels and promoter methylation in renal cancer and thyroid cancer [32]. Consistently, in the present research, we also

discovered that the expression level of *CD3D* was negatively linked to promoter methylation in HNSCC.

Despite the tremendous achievements of multidisciplinary treatment strategies over the past decades, there has been no considerable improvement in the survival rate of individuals with HNSCC, and the 5-year survival rate remains below 50% [7,33]. Therefore, identifying biomarkers that predict patient outcomes is crucial for developing treatment strategies and improving patient survival [34]. The results obtained from the TCGA cohort showed that HNSCC patients with high *CD3D* expression had better overall survival than patients with low expression, which was confirmed in both the GEO and validation cohorts. Concordantly,

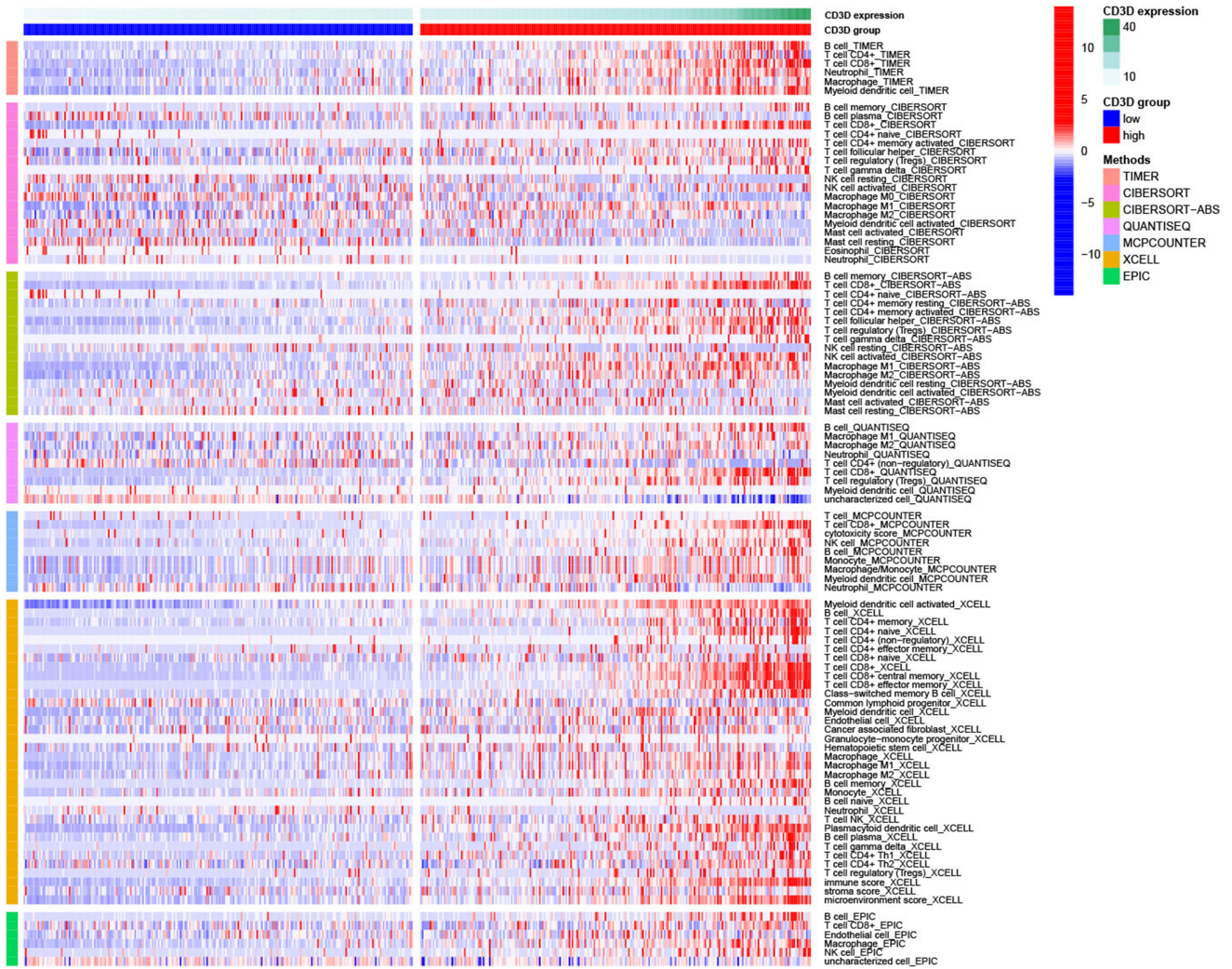


Figure 8. The heatmap shows the relationship between *CD3D* expression and cancer-immune cell infiltration.

univariate and multivariate Cox analysis demonstrated that higher *CD3D* expression independently predicted a better prognosis of patients with HNSCC in the TCGA, GEO, and validation cohorts. However, the AUCs based on only *CD3D* expression showed that the discrimination ability was not satisfactory, indicating that a single biomarker was hardly implemented in clinical application. To optimize the clinical decision-making process for HNSCC, different factors were combined in an integrated biological and clinical model to develop a nomogram and identify biomarkers for more personalized cancer treatment strategies [35,36]. This study constructed a nomogram with important clinical data and *CD3D* expression, which demonstrated

a high prediction performance. Therefore, *CD3D* could be used as a predictive biomarker of the prognosis of HNSCC patients.

Previous research reported that *CD3D* expression was correlated with immune cell abundance in muscle-infiltrating bladder cancer [37]. In the present study, TME analysis confirmed that HNSCC individuals with high *CD3D* expression had higher immune scores and stromal scores. Other immune cell infiltration analyses reported a positive link between the expression levels of *CD3D* and infiltration of various immune effector cells, including B cells, CD4 + T cells, and CD8 + T cells. B cells and T cells are the major players in the adaptive immune response [38]. In

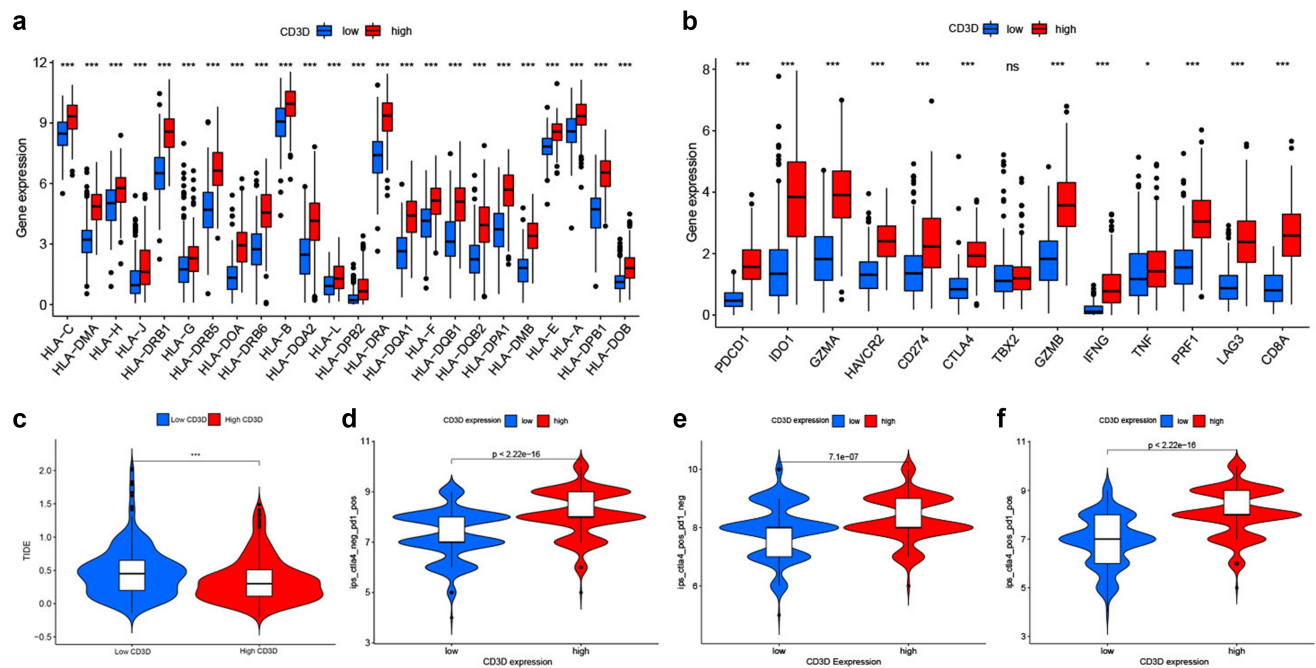


Figure 9. Examination of the predictive significance of *CD3D* in immunotherapy of HNSCC. (a) Human leukocyte antigen (HLA) genes were remarkably expressed in the group with high *CD3D*. (b) ICI-related genes were substantially expressed in the high *CD3D* expression group. (c) The TIDE scores of the group with high *CD3D* expression were remarkably lower than those of the group with low *CD3D* expression. (d) IPS-PD1 markedly increased in the cohort with high *CD3D* expression. (e) IPS-CTLA4 markedly increased in the group with high *CD3D* expression. (f) IPS-CTLA4+ PD1 markedly increased in the group with high *CD3D* expression. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, not significant.

addition, immune cell infiltration is an indication of the host immune response to cancer antigens [39]. Furthermore, functional enrichment analysis demonstrated that *CD3D* plays an essential role in the inflammatory and immune response. The GSEA findings suggested that the high expression of *CD3D* was substantially enriched in antigen processing and presentation, cell adhesion molecules, cytokine–cytokine receptor interaction, and the B receptor signaling pathway. Antigen processing and presentation are essential for a successful humoral response [40]. Increased antigen presentation can lead to adaptive immune responses, including antibody production [41]. In addition, adhesion molecules are critical for immune cell homing to inflamed tissues and lymphoid organs and play an essential role in immune homeostasis in both health and disease [42]. Moreover, cytokine–cytokine receptor interactions and the B-cell receptor signaling pathway are closely associated with the tumor immune

microenvironment and are essential for immune responses in tumors [43]. Moreover, the high *CD3D* expression group was also enriched in the JAK/STAT pathway. The JAK/STAT cytokine signaling pathway has been implicated in proliferative, immunological, and inflammatory responses [44]. Most cytokines involved in immune responses use the JAK/STAT signaling pathway [45]. These findings affirmed that elevated levels of *CD3D* expression might result from the antitumor immune response by recruiting and activating various types of immune cells and may play a defensive function in HNSCC progression, which provides a partial explanation for why high *CD3D* expression in HNSCC predicts a better prognosis.

Despite extensive research on the role of immunotherapy over the past decade, a large proportion of patients with HNSCC still do not respond to approved PD-1/PD-L1-based immunotherapies [46]. Previous clinical trials

demonstrated an overall effectiveness of only 13.3%-17.7% of anti-PD1/PD-L1 immunotherapy in relapsed and metastatic HNSCC [47–49]. Discovering biomarkers that can accurately predict immunotherapy response is a critical step in screening individuals who might gain benefit from immunotherapy. HLA-related genes are among the factors that influence the immunotherapeutic response in patients with tumors [50] and play essential roles in the recognition and destruction of cancerous cells by T lymphocytes [51]. Previous studies have demonstrated that the deletion of HLA-related genes, particularly *HLA-I*, is involved in different biological processes, including tumor progression and immunotherapy resistance [52,53]. Moreover, downregulation of HLA-related genes might contribute to immune evasion [54]. Our study presented a higher level of HLA-related gene expression in the high *CD3D* expression group, which indicated that they had a better immunotherapeutic response than those with low expression of *CD3D*. Indeed, the expression of ICI-related genes is another factor that influences the immunotherapeutic response. Multiple studies have reported that immune checkpoints enhance tumor immune surveillance withdrawal by inhibiting the T-cell response [55]. The results of this research revealed higher expression levels of most ICI-related genes in the high *CD3D* expression group, indicating that these patients could benefit more from immune checkpoint inhibition therapy. Furthermore, our analysis discovered that the high *CD3D* expression group had a lower TIDE score, which suggested that this group had lower tumor immune evasion and achieved more benefits from immune checkpoint inhibition therapy than the low *CD3D* expression group [56]. Consistent with these findings, whether using anti-PD1 and anti-CTLA4 alone or in combination, we found a higher IPS score in patients with high *CD3D* expression, suggesting that they might benefit from immunotherapy both used alone and in combination. Our findings demonstrated that *CD3D* is a favorable predictive biomarker for the immunotherapeutic response in HNSCC.

However, our study has some limitations that need to be noted. First, although we collected samples from public databases and our clinical individuals, the sample size remains modest and requires an extension. Second, we investigated the underlying molecular mechanisms of *CD3D* in HNSCC using GO, KEGG, and GSEA analyses. Additional experiments are required to confirm these findings. Finally, although we used bioinformatic analysis to explore the association between *CD3D* and immune status, as well as immunotherapeutic response, these predictions remain to be experimentally validated.

Conclusion

The present findings suggested that high *CD3D* expression is an independent biomarker of better prognosis in patients with HNSCC and is associated with immune cell infiltration. Additionally, patients with high *CD3D* expression levels might respond better to immunotherapy. However, studies with larger samples and further experiments are required to verify our results.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data supporting this study’s findings are available from The Cancer Genome Atlas (TCGA) at <https://portal.gdc.cancer.gov/> and the Gene-Expression Omnibus (GEO) at <https://www.ncbi.nlm.nih.gov/geo/>.

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