



PLCXD2 expression relates to the immune microenvironment and prognosis of head and neck squamous cell carcinoma: a retrospective cohort study

Mingming Tang^{1,2,3#}, Qingwen Chen^{1,2,3#^}, Xinjiang Xu^{1,2}, Zihao Zhang^{1,2}, Liang Han^{1,2^}, Hao Wu^{3^}

¹Department of Head and Neck Surgery, Nantong Tumor Hospital/Affiliated Tumor Hospital of Nantong University, Nantong, China; ²Department of Otolaryngology, Medical School of Nantong University, Nantong, China; ³Department of Otorhinolaryngology Head and Neck Surgery, Affiliated Hospital of Nantong University, Nantong, China

Contributions: (I) Conception and design: L Han; (II) Administrative support: H Wu; (III) Provision of study materials or patients: M Tang; (IV) Collection and assembly of data: Q Chen; (V) Data analysis and interpretation: M Tang, Q Chen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Hao Wu, MD. Department of Otorhinolaryngology Head and Neck Surgery, Affiliated Hospital of Nantong University, Xisi Road No. 20, Nantong 226000, China. Email: entwuhao@163.com; Liang Han, MD. Department of Head and Neck Surgery, Nantong Tumor Hospital/Affiliated Tumor Hospital of Nantong University, Tongyang Road No. 30, Nantong 226000, China; Department of Otolaryngology, Medical School of Nantong University, Qixiu Road No. 19, Nantong 226000, China. Email: hl61697@126.com.

Background: Despite the advances in oncology, the prognosis of head and neck squamous cell carcinoma (HNSC) patients remains dismal. The limited response rates to immune checkpoint inhibitors highlight the urgent need for novel therapeutic targets. In this study, we aimed to determine the relevance of PLCXD2 expression in the tumor microenvironment to the HNSC patient clinicopathological features.

Methods: Gene expression analysis and multicolor immunofluorescence histochemistry with HNSC tissue microarrays were conducted to examine the relation between PLCXD2 expression and patient outcomes. We retrospectively analyzed 275 treatment-naïve patients who underwent surgery for HNSC from 2004–2013. Baseline clinicopathological data, and tumor stage, were retrieved from medical records. The primary prognostic outcome was five-year overall survival, with data censored at the last follow-up for patients who were still alive. Additionally, Spearman correlation analysis was used to assess the relationship between PLCXD2 protein expression and tumor immune infiltrating cells (TIICs), as well as immune checkpoints [programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1) and CTLA-4] in HNSC tissue, while chi-squared test and Cox proportional-hazards models were employed to validate the correlation between PLCXD2 protein levels and clinicopathological characteristics with patient survival.

Results: Our findings revealed higher PLCXD2 protein expression in cancer cells of HNSC tissue compared to control benign tissues ($Z=-3.890$, $P<0.001$). Additionally, we observed a distinct association between the presence of PLCXD2 protein in cancer nests and various TIICs, including CD4⁺ T cells, CD8⁺ T cells, dendritic cells, as well as CTLA-4⁺ cells in HNSC tissues. Furthermore, we demonstrated a correlation between PLCXD2 protein expression in cancer cells (hazard ratio: 1.955, $P=0.01$) and advanced TNM stage (hazard ratio: 1.617, $P=0.001$), as well as a poorer prognosis.

Conclusions: Our findings demonstrate that elevated PLCXD2 expression in HNSC is significantly associated with advanced tumor stage and poorer patient prognosis. The correlation of PLCXD2 with key tumor-infiltrating immune cells and the CTLA-4 checkpoint implicates its role in modulating the tumor immune microenvironment. Therefore, this study supports PLCXD2 as an independent prognostic marker and a potentially promising target for immunotherapy in HNSC.

[^] ORCID: Liang Han, 0000-0002-0335-1848; Qingwen Chen, 0009-0003-4602-4702; Hao Wu, 0000-0003-0324-8215.

Keywords: PLCXD2; head and neck squamous cell carcinoma (HNSC); tumor immune infiltrating cells (TIICs); CTLA-4; prognosis

Submitted Apr 26, 2025. Accepted for publication Aug 29, 2025. Published online Oct 29, 2025.

doi: 10.21037/tcr-2025-880

View this article at: <https://dx.doi.org/10.21037/tcr-2025-880>

Introduction

Head and neck cancers rank seventh among malignant tumors worldwide (1,2), with over 90% of these malignancies being head and neck squamous cell carcinomas (HNSC) originating from the mucosal surfaces in this region (1,2). While surgery, radiotherapy and chemotherapy remain the primary treatment modalities as they can enhance patient prognosis in clinical settings, advanced cases still exhibit a 50% recurrence rate with life-threatening consequences (3). This high recurrence rate can be attributed, in part, to the complex tumor microenvironment (TME), which comprises various tumor immune infiltrating cells (TIICs) and other components alongside cancer cells and stromal cells (4). Based on research on the TME in recent literature, emerging immune checkpoint inhibitors (ICIs) have shown promise in improving outcomes for select patients (3,5). To leverage the TME for prognosis and treatment, multiple biomarkers have been intensively investigated in HNSC. Most notably,

programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1) and CTLA-4 expression have been used to guide ICIs therapy, while the density of TIICs and HPV status have been identified as key prognostic factors (6,7). Similarly, while a high density of TIICs is generally beneficial, it fails to capture the functional state of these cells, which can become exhausted and ineffective in the immunosuppressive TME (8). This suggests that single-analyte biomarkers are often insufficient to capture the complex dynamic interactions occurring within tumors. Therefore, there is a critical need to identify more effective therapeutic targets in clinical practice (9,10).

The Cancer Genome Atlas (TCGA) utilizes high-throughput next-generation sequencing (NGS) and bioinformatics to provide gene expression data for 33 tumor types (11), including HNSC (12). This freely accessible data resource has significantly enhanced the capabilities of the tumor research community in diagnosing, treating, and predicting outcomes for cancer patients (11). Additionally, the Genotype-Tissue Expression (GTEx) portal serves as a comprehensive web-based platform for researchers to investigate cell- and tissue-specific gene expression patterns (13,14). Using these two databases, our preliminary assessments revealed significantly elevated phosphatidylinositol-specific phospholipase C X domain containing 2 (PLCXD2) messenger RNA (mRNA) expression in HNSC tissue compared to non-tumor tissues and its association with patient prognosis.

PLCXD2, a protein-coding gene located on chromosome 3q13.2, was found to exhibit higher mRNA expression in HNSC tissue compared to non-tumor tissues and was associated with patient prognosis. Functionally, PLCXD2 is predicted to facilitate phosphoric diester hydrolase activity and participates in lipid catabolic processes and signal transduction pathways (15). Notably, PLCXD2 protein expression was predominantly detected in the cell nucleus of HeLa cell lines (16). Despite these insights, PLCXD2 clinical significance in HNSC cancer tissues remains poorly understood. While many research teams have relied on gene expression data to infer protein expression changes (17), it is widely recognized that the correlation between mRNA and

Highlight box

Key findings

- This work investigates the relevance of PLCXD2 expression in the tumor microenvironment (TME) to the head and neck squamous cell carcinoma (HNSC) patient clinicopathological features.

What is known and what is new?

- High PLCXD2 protein expression in cancer cells correlated with advanced TNM stage, lymph node metastasis, and poorer overall survival, identifying it as an independent prognostic marker. Additionally, PLCXD2 expression in cancer cells was positively associated with tumor immune infiltrating cells such as CD4⁺ T cells, CD8⁺ T cells, dendritic cells, and CTLA-4⁺ cells, suggesting its involvement in the TME and potential as an immunotherapeutic target.

What is the implication, and what should change now?

- This work emphasizes PLCXD2 as an indicator for prognosis and a promising candidate for immunotherapeutic interventions in HNSC. We need functional experiments using cellular or animal models to elucidate the mechanistic role of PLCXD2 in HNSC tumorigenesis and its interactions within the TME.

protein expression levels is often weak (17,18).

In this present study, we employed multicolor immunofluorescence histochemistry (mIHC) utilizing HNSC tissue microarray (TMA) specimens and clinical data to assess PLCXD2 protein expression in the TME and examine its correlation with immune checkpoint molecules. Collectively, the results emphasize PLCXD2 as an indicator for prognosis and a promising candidate for immunotherapeutic interventions in HNSC. We present this article in accordance with the REMARK reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-880/rc>).

Methods

Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA2 (<http://gepia2.cancer-pku.cn/#index>) can be used to analyze bulk gene expression profiles of tumors and benign tissues using data from the TCGA and GTEx datasets (19). In this study, this bioinformatics tool was used to compare PLCXD2 mRNA expression levels in HNSC tissues (n=519) with those in benign control tissues (n=44). Additionally, we assessed the relationship of PLCXD2 mRNA expression with HNSC patients' clinical outcomes.

Clinical samples and information

TMAs comprising samples from various sites within the head and neck region, including 113 tongue cancer tissues, 86 buccal mucosa cancerous tissues, 76 larynx carcinoma tissues, 55 matched non-cancerous tissues from surgical margins and 6 squamous papillomae, and the clinical and follow-up data of these corresponding patients were obtained from the Affiliated Hospital of Nantong University for the period from year 2004 to 2013. The TMA cores contained tissues with a diameter of 2 mm, and the section thickness of the TMA was 4 microns, as previously described (20,21). Baseline clinicopathological factors, such as gender, age, tumor location, differentiation grade and tumor stage according to the 8th edition of the American Joint Committee on Cancer (AJCC) classification (22), were collected from the patients' medical records. Importantly, none of the included patients in the study had undergone radiotherapy, chemotherapy or immunotherapy prior to surgery. The primary prognostic outcome was five-year overall survival (OS), with data censored at the last follow-up for patients who were still alive. The study protocol was

approved by the Human Research Ethics Committee of the Affiliated Hospital of Nantong University (No. 2018-K020). This study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The individual consent for this retrospective analysis was waived.

mIHC staining and scoring

We conducted mIHC staining using the Opal 7-Color mIHC Kit (NEL810001KT; Akoya Biosciences, USA). Antigen retrieval on the TMA slides was achieved through microwave heating, following the kit's instructions. After incubating the primary antibodies (refer to [Table S1](#)) and subsequent washing steps, the sections were further treated with Opal™ secondary-HRP and underwent additional washes, and this cycle was repeated as necessary, following which nuclear staining was performed on the TMA sections using DAPI (F6057, Sigma, USA) according to established protocols (23,24).

The multicolor immunofluorescence-stained TMA sections were scanned using Multispectral Imaging and Whole Slide Scanning (Vectra 3.0, PerkinElmer, USA). The differential positive fluorescence signals of various proteins (including PLCXD2 and other immune markers) in each tissue core were individually assessed within the tumor and stroma using matched software (inForm 2.6, PerkinElmer, USA). The final score, ranging from 0 to 100, was calculated as the ratio of stained cells to the number of Cytokeratin or DAPI multiplied by 100%.

Statistical analysis

Wilcoxon rank-sum test and Wilcoxon Signed Ranks test were used to compare PLCXD2 protein expression levels in the TME of HNSC and non-tumorous tissues. Pearson's test was utilized for assessing the correlation between PLCXD2 protein levels and other immune markers. The X-tile tool (Yale University, USA) was used to determine the optimal cutoff point for PLCXD2 protein expression based on patients' OS rates. The association between PLCXD2 protein expression and clinicopathological features was assessed using chi-squared test. Prognostic factors for HNSC were determined using Cox proportional-hazards models and the Kaplan-Meier (K-M) method. To identify independent prognostic factors, only variables demonstrating statistical significance in univariate analysis were included in the subsequent multivariate analysis.

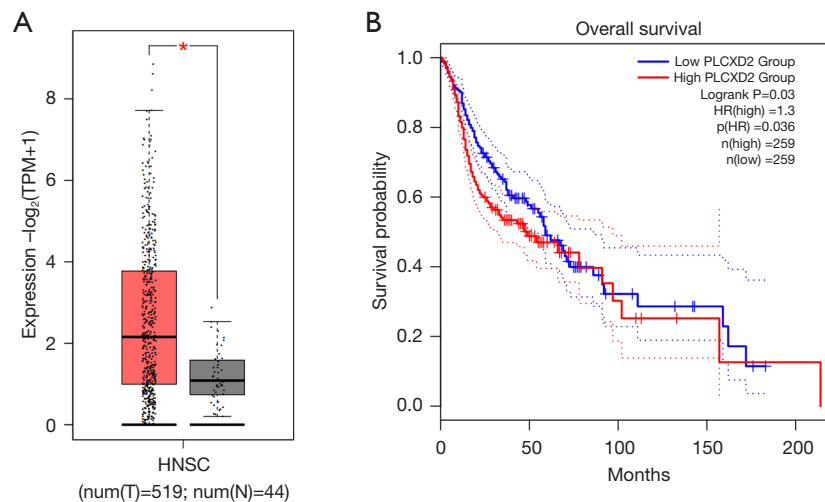


Figure 1 GEPIA analysis of PLCXD2 mRNA in HNSC tissues. (A) Differential expression of PLCXD2 mRNA in HNSC (red) compared to benign tissue (gray). (B) Survival curve of PLCXD2 mRNA expression in HNSC patients. GEPIA, Gene Expression Profiling Interactive Analysis; HNSC, head and neck squamous cell carcinoma; HR, hazard ratio; mRNA, messenger RNA; TPM, transcripts per million.

Statistical analyses were performed using SPSS 24.0 (IBM, USA). All statistical tests were two-sided, and a significance level was set at $P < 0.05$. The significance threshold of R was > 0.2 .

Results

PLCXD2 mRNA expression in HNSC tissues

Here, we assessed the feasibility of PLCXD2 as a biomarker for HNSC. As anticipated, we found that PLCXD2 mRNA expression in HNSC tissue was significantly elevated compared to non-cancerous tissues, with a notable statistical difference ($\log_2FC < 1$, $P < 0.01$) (Figure 1A). Additionally, high PLCXD2 mRNA expressions were strongly associated with poorer prognoses [hazard ratio (HR) = 1.3, $P = 0.03$] in patients with HNSC (Figure 1B).

Association between PLCXD2 protein levels and immune markers in HNSC tissues TME

We conducted mIHC staining on HNSC TMA sections to analyze the relationship between PLCXD2 protein expression and various TIICs, including CD4⁺ T cells, CD8⁺ T cells, CD56⁺ NK cells, CD66b⁺ neutrophils, CD68⁺CD86⁺ M1-like macrophages, CD68⁺CD163⁺ M2-like macrophages and Lamp3⁺ dendritic cells, and also examined their correlation with commonly used immune checkpoints PD-1, PD-L1, and CTLA-4.

PLCXD2 protein was primarily localized in the nuclear and cytoplasmic compartments of both HNSC tissues and benign squamous epithelial tissues. Notably, PLCXD2 protein expression varied across different cell types, being observed not only in cancer cells and non-tumor squamous epithelial cells but also in stromal cells. Wilcoxon rank sum test revealed that PLCXD2 protein expression was significantly higher in cancer cells (10.58 ± 14.07) compared to squamous epithelial cells (4.16 ± 5.24) ($Z = -3.890$, $P < 0.001$). Additionally, PLCXD2 protein expression in cancer stromal cells (21.51 ± 21.39) was significantly higher than in benign stromal cells (12.14 ± 13.24) ($Z = -3.890$, $P = 0.007$) (Figure 2A,2B). These results aligned with the PLCXD2 mRNA expression findings from the GEPIA data. Furthermore, the Wilcoxon Signed Ranks Test demonstrated that PLCXD2 protein expression in cancer cells was significantly lower than in cancer stromal cells ($Z = -6.498$, $P < 0.001$).

We analyzed PLCXD2 protein expression in the TME of HNSC using Pearson correlation analysis and observed a correlation between PLCXD2 protein levels in cancer nests and that in cancer stromal cells ($r = 0.216$, $P < 0.001$). (Figure 2C). We observed CTLA-4 protein expression not only in TIICs but also in some cancer cells. (Figure 2D). Additionally, PLCXD2 protein levels in cancer cells were positively correlated with CD4⁺ T cells (CD3⁺CD4⁺) ($r = 0.236$, $P < 0.001$), CD8⁺ T cells (CD3⁺CD8⁺) in the cancer stroma ($r = 0.313$, $P < 0.001$), and dendritic cells (Lamp3⁺) in

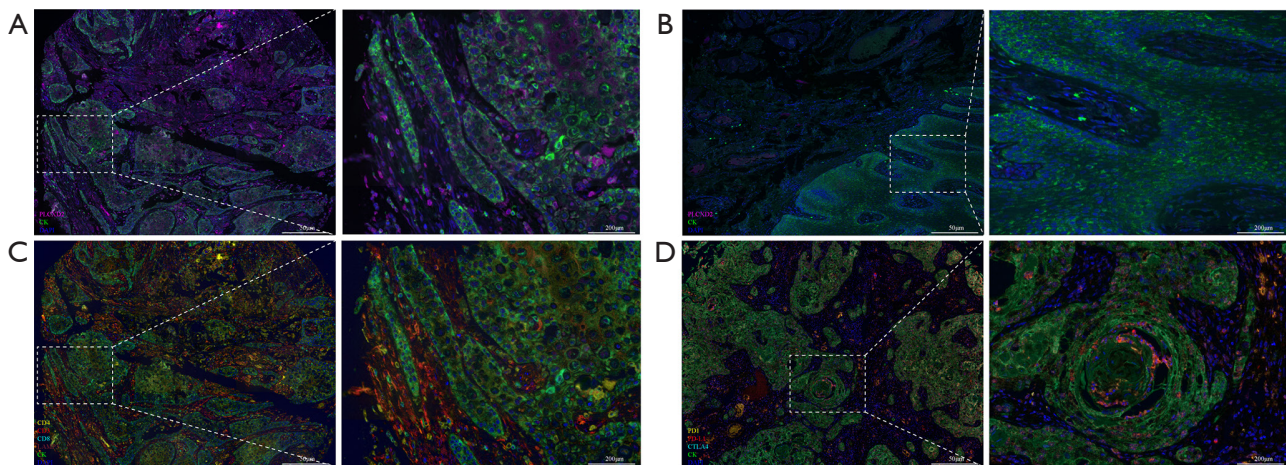


Figure 2 Representative images depicting PLCXD2 protein expression and immune markers in HNSC tissue. Higher expression and lower expression of PLCXD2 protein in HNSC (A) compared to non-tumor squamous epithelial tissue (B). PLCXD2 protein expression is shown in purple. (C) Immunostaining for CD3⁺ cells, CD4⁺ cells, and CD8⁺ cells in HNSC tissue. (D) Immunostaining for Lamp3⁺ cells and CTLA-4⁺ cells in HNSC tissue. Cytokeratin staining is shown in green, and nuclei are stained with DAPI (blue). HNSC, head and neck squamous cell carcinoma.

both intratumoral ($r=0.204$, $P=0.008$) and stroma ($r=0.212$, $P=0.005$). (Figure 3A, Table S2). PLCXD2 protein levels in cancer cells were correlated with CTLA-4⁺ cells in cancer nests ($r=0.262$, $P<0.001$). However, no significant correlation was found between PLCXD2 protein expression in cancer stromal cells and TIICs or immune checkpoints (PD-1, PD-L1, and CTLA-4) in the TME of HNSC (Figure 3B, Table S3).

Association between PLCXD2 protein levels and clinicopathological characteristics of HNSC patients

Based on the OS of HNSC patients, we determined a cutoff point for PLCXD2 protein expression in cancer cells (score, 0–91.97). Scores equal to or less than 8.57 were classified as low-expression ($n=167$), while scores ranging from 8.57 to 100 were categorized as high-expression ($n=108$) groups. Chi-square test results confirmed that PLCXD2 protein expression in cancer cells was associated with age ($\chi^2=4.331$, $P=0.03$), tumor location ($\chi^2=12.261$, $P=0.002$), depth of tumor invasion ($\chi^2=13.369$, $P=0.01$), lymph node metastasis (LNM) ($\chi^2=5.041$, $P=0.02$), and TNM classification ($\chi^2=15.543$, $P=0.004$) (Table 1).

However, no suitable cutoff value was identified to establish a relationship between the score of PLCXD2 protein expression in stromal cells (ranging from 0 to 92.59) and clinical outcomes. Even when considering the

median or half of the population, no evidence was found to suggest a correlation between PLCXD2 protein expression in stromal cells and clinical features of HNSC (data not shown).

The relation between the PLCXD2 protein expression and HNSC patient prognosis

To determine the relationship between PLCXD2 protein status and 5-year OS in patients, Cox regression analysis and K-M curves with log-rank tests were conducted. Univariate analysis revealed that PLCXD2 protein expression (HR: 2.232, $P=0.001$), tumor differentiation (HR: 1.532, $P=0.03$), depth of tumor invasion (HR: 1.482, $P=0.005$), LNM (HR: 3.314, $P<0.001$) and TNM classification (HR: 1.754, $P<0.001$) significantly influenced patient survival. In multivariate analyses, PLCXD2 protein expression (HR: 1.955, $P=0.01$) and TNM classification (HR: 1.617, $P=0.001$) were identified as independently associated with the 5-year OS of HNSC patients (Table 2, Figure 4).

Discussion

This study utilized bioinformatics to investigate the association between PLCXD2 mRNA expression and patient prognosis in HNSC. Upon validation using mIHC, PLCXD2 protein expression was found to be correlated

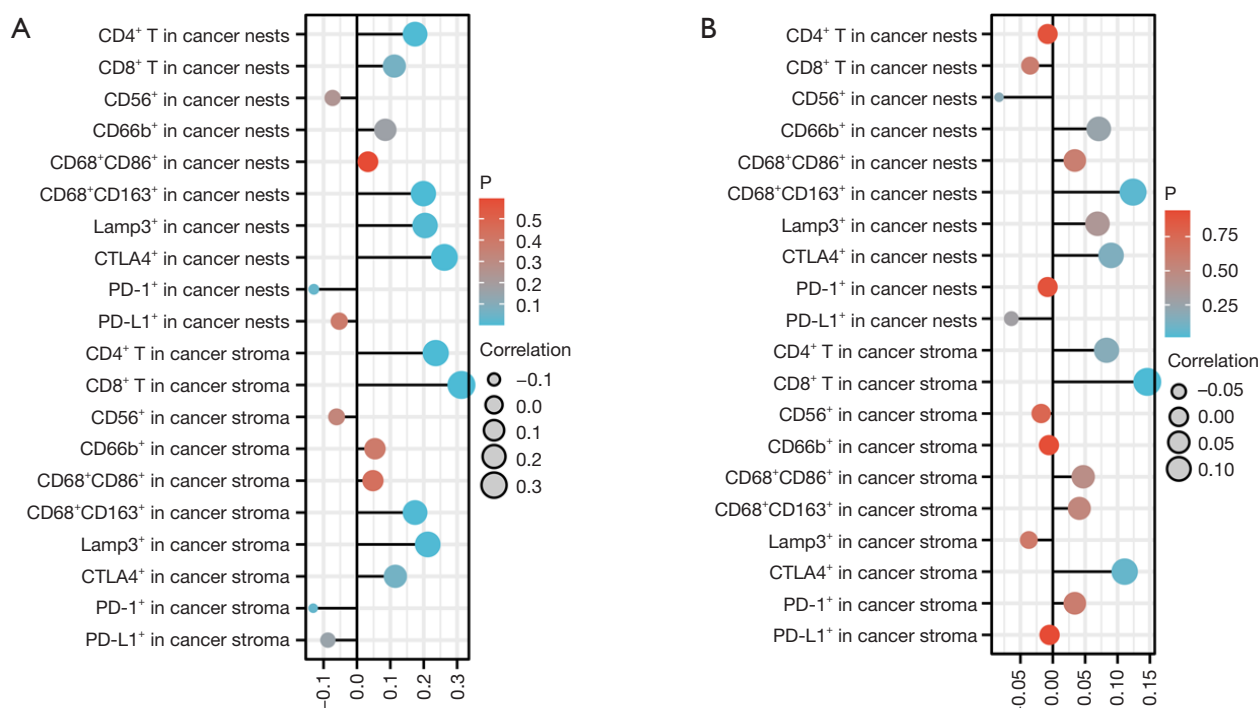


Figure 3 The relationship between PLCXD2 protein expression and immune markers in the TME of HNSC tissues. (A) The relationship between PLCXD2 protein expression in cancer cells and immune markers in the TME of HNSC tissues. (B) The relationship between PLCXD2 protein expression in stroma cells and immune markers in the TME of HNSC tissues. HNSC, head and neck squamous cell carcinoma; TME, tumor microenvironment.

with tumor malignancy and the status of the TME. Further analysis of PLCXD2 at the protein expression level revealed its relevance to tumor progression and identified it as an independent prognostic factor in HNSC.

Previous studies investigating the relationship between the PLCXD2 gene and human cancer through Genome-wide association studies (GWAS) have demonstrated that PLCXD2 (specifically rs2399395 at 3q13.2) is associated with the risk of esophageal squamous cell carcinoma and lung cancer in the Chinese Han populations (25,26). However, these studies did not explore correlations between the TME and clinical characteristics at the PLCXD2 protein expression level. Given that mRNA expression by different tissue cells can result in the production of proteins with unique biological activities and diverse physiological functions (18,27), we investigated PLCXD2 protein expression in the TME of HNSC tissues.

As anticipated, PLCXD2 protein expression was predominantly observed in the nucleus of both benign and tumor cells, with varying degrees of expression. Importantly, the presence of PLCXD2 protein in HNSC tissue was

significantly elevated compared to non-cancerous tissues. Additionally, we found a correlation between PLCXD2 protein expression in cancer nests and that in cancer stromal cells. Considering our observation that high expression of PLCXD2 was closely associated with poor prognosis in HNSC patients, we speculate that PLCXD2 may play a role in promoting the occurrence and development of malignant tumors.

The complicated TME encompasses cancer cells, stromal cells (including TIICs, carcinoma-associated fibroblasts, and other cell types), and the extracellular matrix (28). Understanding the TME is crucial for advancing cancer immunoprevention and immune interception strategies (29). Thus, we conducted a correlation analysis between the score of PLCXD2 protein expression and the identified TIICs or immune checkpoints in the TMA of HNSC.

Our findings revealed a positive correlation between PLCXD2 protein expression in cancer cells and the presence of CD4⁺ T cells and CD8⁺ T cells in the cancer stroma, as well as dendritic cells in both the cancer intratumoral area and stroma. Furthermore, PLCXD2

Table 1 The association between PLCXD2 protein expression in cancer cells and clinical characteristics of HNSC

Characteristics	N	PLCXD2 protein in cancer cells		χ^2	P value
		Low expression, n (%)	High expression, n (%)		
Total	275	167 (60.73)	108 (39.27)		
Age (years)				4.331	0.03*
≤60	118	80 (67.80)	38 (32.20)		
>60	157	87 (55.41)	70 (44.59)		
Gender				0.677	0.41
Male	105	67 (63.81)	38 (36.19)		
Female	170	100 (58.82)	70 (41.18)		
Location				12.261	0.002*
Tongue	113	81 (71.68)	32 (28.32)		
Buccal mucosa	86	51 (59.30)	35 (40.70)		
Larynx	76	35 (46.05)	41 (53.95)		
Differentiation				1.134	0.56
Well	150	94 (62.67)	56 (37.33)		
Middle	115	66 (57.39)	49 (42.61)		
Poor	10	7 (70.00)	3 (30.00)		
T stage				13.369	0.01*
Tis	14	12 (85.71)	2 (14.29)		
I	74	51 (68.92)	23 (31.08)		
II	123	72 (58.54)	51 (41.46)		
III	45	24 (53.33)	21 (46.67)		
IV	7	1 (14.29)	6 (85.71)		
Unknown	12				
Lymph node metastasis				5.041	0.02*
Yes	51	24 (47.06)	27 (52.94)		
No	212	136 (64.15)	76 (35.85)		
Unknown	12				
TNM stage				15.543	0.004*
Stage 0	18	14 (77.78)	4 (22.22)		
Stage I	60	42 (70.00)	18 (30.00)		
Stage II	94	59 (62.77)	35 (37.23)		
Stage III	82	44 (53.66)	38 (46.34)		
Stage IV	9	1 (11.11)	8 (88.89)		
Unknown	12				

*, P<0.05. HNSC, head and neck squamous cell carcinoma.

Table 2 Univariate and multivariate analysis of factors associated with HNSC patient survival

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	P > z	HR	95% CI	P > z
PLCXD2 protein expression in cancer cells (high vs. low)	2.332	1.426–3.814	0.001*	1.955	1.175–3.253	0.01*
Age (≤60 vs. >60 years)	1.587	0.949–2.653	0.07			
Gender (male vs. female)	1.441	0.844–2.460	0.18			
Location (tongue vs. buccal mucosa)	1.125	0.839–1.508	0.43			
Differentiation (well and middle vs. poor)	1.532	1.038–2.261	0.03*	1.475	0.994–2.188	0.054
T (Tis vs. T2 vs. T3 vs. T4)	1.482	1.125–1.953	0.005*			
Lymph node metastasis (yes vs. no)	3.314	1.993–5.510	<0.001*			
TNM stage (0 vs. I vs. II vs. III and IV)	1.754	1.333–2.309	<0.001*	1.617	1.219–2.146	0.001*

*, P<0.05. CI, confidence interval; HNSC, head and neck squamous cell carcinoma; HR, hazard ratio.

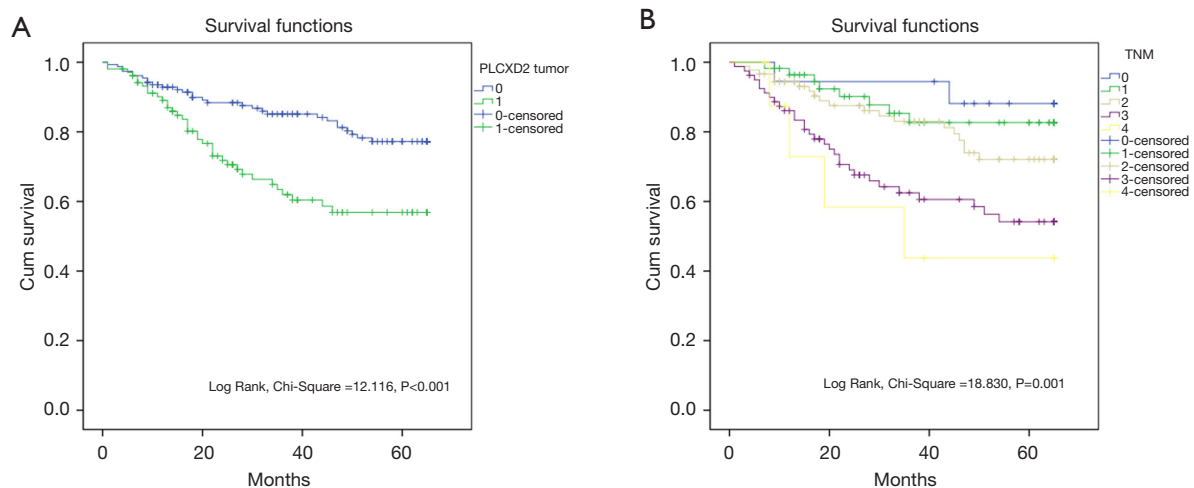


Figure 4 Relationship between PLCXD2 protein expression and immune markers in the TME of HNSC tissues. (A) PLCXD2 protein expression in cancer cells. (B) PLCXD2 protein expression in stromal cells and immune markers. HNSC, head and neck squamous cell carcinoma; TME, tumor microenvironment.

protein expression in cancer cells was associated with CTLA-4⁺ cells in nests. Taken together, these results indicate a positive linear dependence between PLCXD2 protein expression and the presence of these immune cell populations.

Within the TME, CD4⁺ T cells exhibit diverse functions in cancer surveillance and are linked to varying outcomes (30). CD8⁺ T cells serve as cytotoxic T cells, binding to MHC-I antigens and exerting anti-cancer effects (31,32). Dendritic cells, as crucial antigen-presenting cells in the TME, integrate innate and adaptive immunity and activate T cells (33,34). CTLA-4, an important

immune checkpoint, is upregulated in activated T cells and suppresses cancer immune responses (35,36). Recent studies have consistently shown enhanced CTLA-4 protein expression in tumor cells of various human cancer tissues (37–39), consistent with our findings and indicating a potential association with poor patient prognosis. Based on our correlation analysis results, increased PLCXD2 protein expression in cancer tissue may stimulate the secretion of specific cytokines and recruit TIICs such as CD4⁺ T cells, CD8⁺ T cells and dendritic cells. Concurrently, it may contribute to an increase in the ratio of CTLA-4⁺ cells within the TME.

The observed phenomenon suggests that the facilitating effect of PLCXD2 protein on cancer progression may outweigh the tumor immunity effect exerted by these three types of TIICs in the TME of HNSC. Given that clinical application of monoclonal antibody immunosuppressants targeting CTLA-4 has been shown to enhance the effectiveness of HNSC treatment (40), it could be beneficial to explore the use of anti-CTLA-4 monoclonal antibodies to improve treatment outcomes in cases where PLCXD2 protein expression is high in HNSC tissues.

HNSC, categorized as a solid tumor, exhibits TNM classifications closely associated with patient prognosis (41). In this study, we investigated aberrant PLCXD2 protein expression in relation to clinical characteristics and TNM staging. High PLCXD2 protein expression in cancer cells has been commonly reported in older patients, with tumors located predominantly in the larynx, followed by the buccal mucosa and tongue. Additionally, elevated PLCXD2 protein expression correlated with increased tumor depth invasion, regional LNM, and advanced TNM classification, all of which were associated with poorer prognosis. This suggests that up-regulation of PLCXD2 protein in cancer cells may accelerate HNSC progression. Our findings suggest that PLCXD2 may serve as a candidate oncogene facilitating malignant tumor development and could potentially serve as a prognostic marker for HNSC patients.

Our research has several limitations. Firstly, further investigation into the role of PLCXD2 in tumorigenesis is warranted using cellular and animal models. Secondly, mechanistic experiments are needed to elucidate immune interactions within the TME of HNSC. Lastly, the lack of multicenter HNSC tissue samples limits the generalizability of our research findings.

Conclusions

In conclusion, our study demonstrates that elevated PLCXD2 expression in HNSC is significantly associated with advanced tumor stage and adverse patient prognosis. The observed correlation between PLCXD2 levels and key tumor-infiltrating immune cell populations, as well as the immune checkpoint molecule CTLA-4, underscores its potential role in modulating the tumor immune microenvironment. Moreover, our findings establish PLCXD2 protein expression in HNSC tissues as an independent prognostic biomarker and highlight it as a promising candidate for immunotherapeutic intervention.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-880/rc>

Data Sharing Statement: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-880/dss>

Peer Review File: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-880/prf>

Funding: This work was supported by the Science and Technology Project of Nantong City (No. JC2023082 to L.H., No. JCZ2022069 to Q.C.); Nantong Municipal Commission of Health and Family Planning (No. MS2023064 to M.T., No. QNZ2023057 to Q.C.); Natural Science Foundation of Nantong University (No. 2022JQ003 to Q.C.); Affiliated Hospital of Nantong University Research Physician Development Fund (No. YJXY202204-YSB02 to H.W.).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-880/coif>). M.T. reports the funding from Nantong Municipal Commission of Health and Family Planning (No. MS2023064). Q.C. reports the funding from Science and Technology Project of Nantong City (No. JCZ2022069); Nantong Municipal Commission of Health and Family Planning (No. QNZ2023057); and Natural Science Foundation of Nantong University (No. 2022JQ003). L.H. reports the funding from the Science and Technology Project of Nantong City (No. JC2023082). H.W. reports the funding from the Affiliated Hospital of Nantong University Research Physician Development Fund (No. YJXY202204-YSB02). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki

and its subsequent amendments. The study protocol was approved by the Human Research Ethics Committee of the Affiliated Hospital of Nantong University (No. 2018-K020). The individual consent for this retrospective analysis was waived.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Tang Y, Hu T, Yin W, et al. Bioinformatics analysis reveals VEGFC's prognostic significance in head and neck squamous cell carcinoma and its association with immune cell infiltration. *Transl Cancer Res* 2024;13:5953-70.
2. Wang S, Jiang J, Xing M, et al. Deciphering the prognostic potential of a necroptosis-related gene signature in head and neck squamous cell carcinoma: a bioinformatic analysis. *Transl Cancer Res* 2025;14:340-53.
3. Smussi D, Mattavelli D, Paderno A, et al. Revisiting the concept of neoadjuvant and induction therapy in head and neck cancer with the advent of immunotherapy. *Cancer Treat Rev* 2023;121:102644.
4. Qiu X, Lin Y, Li Q, et al. Integrated single-cell and bulk RNA sequencing reveals prognostic and immunotherapy-associated myofibroblastic cancer-associated fibroblast subtypes in head and neck squamous cell carcinoma. *Transl Cancer Res* 2025;14:3730-45.
5. Wang Z, Zhao Y, Zhang L. Emerging trends and hot topics in the application of multi-omics in drug discovery: a bibliometric and visualized study. *Current Pharmaceutical Analysis* 2024;21:20-32.
6. Cao F, Li Y, Fang Q, et al. Cadonilimab (a PD-1/CTLA-4 Bispecific Antibody) plus Neoadjuvant Chemotherapy in Locally Advanced Head and Neck Squamous Cell Carcinoma: A Phase II Clinical Trial. *Clin Cancer Res* 2025;31:3876-85.
7. Li H, Zandberg DP, Kulkarni A, et al. Distinct CD8(+) T cell dynamics associate with response to neoadjuvant cancer immunotherapies. *Cancer Cell* 2025;43:757-775.e8.
8. Zandberg DP, Vujanovic L, Clump DA, et al. Randomized Phase II Study of Concurrent Versus Sequential Pembrolizumab in Combination With Chemoradiation in Locally Advanced Head and Neck Cancer. *J Clin Oncol* 2025;43:2572-82.
9. Olmedo I, Martínez D, Carrasco-Rojas J, et al. Mitochondria in oral cancer stem cells: Unraveling the potential drug targets for new and old drugs. *Life Sci* 2023;331:122065.
10. Bao J, Betzler AC, Hess J, et al. Exploring the dual role of B cells in solid tumors: implications for head and neck squamous cell carcinoma. *Front Immunol* 2023;14:1233085.
11. Moiso E, Farahani A, Marble HD, et al. Developmental Deconvolution for Classification of Cancer Origin. *Cancer Discov* 2022;12:2566-85.
12. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 2015;517:576-82.
13. Guo Y, Zhang R, Xu H, et al. Pan-cancer Multi-omics Analysis Reveals HMGN1 as a Potential Prognostic and Immune Infiltration-associated Biomarker. *Curr Med Chem* 2025;32:2440-59.
14. Rockweiler NB, Ramu A, Nagirnaja L, et al. The origins and functional effects of postzygotic mutations throughout the human life span. *Science* 2023;380:eabn7113.
15. Harmonizing model organism data in the Alliance of Genome Resources. *Genetics* 2022;220:iyac022.
16. Gellatly SA, Kalujnaia S, Cramb G. Cloning, tissue distribution and sub-cellular localisation of phospholipase C X-domain containing protein (PLCXD) isoforms. *Biochem Biophys Res Commun* 2012;424:651-6.
17. Maier T, Güell M, Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett* 2009;583:3966-73.
18. Wegler C, Ölander M, Wiśniewski JR, et al. Global variability analysis of mRNA and protein concentrations across and within human tissues. *NAR Genom Bioinform* 2020;2:lqz010.
19. Li C, Tang Z, Zhang W, et al. GEPIA2021: integrating multiple deconvolution-based analysis into GEPIA. *Nucleic Acids Res* 2021;49:W242-6.
20. Tang Z, Li J, Lu B, et al. CircBIRC6 facilitates the malignant progression via miR-488/GRIN2D-mediated CAV1-autophagy signal axis in gastric cancer. *Pharmacol Res* 2024;202:107127.
21. Abudusalam K, Xu Y, Keyumu P, et al. WSCD2 Expression: Its Relevance to Tumor-Infiltrating Immune Cells and Glioma Prognosis. *Curr Med Chem* 2025;32:5043-52.

22. Bhateja P, Bonomi M, Verschraegen C. Novel combinations with programmed cell death 1 inhibitor for incurable recurrent/metastatic head and neck squamous cell carcinoma (RM HNSCC): is cabozantinib a front runner? *Transl Cancer Res* 2025;14:2548-52.
23. Sun P, Zhang H, Shi J, et al. KRTCAP2 as an immunological and prognostic biomarker of hepatocellular carcinoma. *Colloids Surf B Biointerfaces* 2023;222:113124.
24. Lu B, Lu T, Shi J, et al. Basic Transcription Factor 3 Like 4 Enhances Malignant Phenotypes through Modulating Tumor Cell Function and Immune Microenvironment in Glioma. *Am J Pathol* 2024;194:772-84.
25. Jin G, Ma H, Wu C, et al. Genetic variants at 6p21.1 and 7p15.3 are associated with risk of multiple cancers in Han Chinese. *Am J Hum Genet* 2012;91:928-34.
26. Wang J, Wang Q, Wei B, et al. Intronic polymorphisms in genes LRFN2 (rs2494938) and DNAH11 (rs2285947) are prognostic indicators of esophageal squamous cell carcinoma. *BMC Med Genet* 2019;20:72.
27. Liu Y, Beyer A, Aebersold R. On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell* 2016;165:535-50.
28. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* 2015;348:74-80.
29. Stanton SE, Castle PE, Finn OJ, et al. Advances and challenges in cancer immunoprevention and immune interception. *J Immunother Cancer* 2024;12:e007815.
30. Bawden E, Gebhardt T. The multifaceted roles of CD4(+) T cells and MHC class II in cancer surveillance. *Curr Opin Immunol* 2023;83:102345.
31. Giles JR, Globig AM, Kaech SM, et al. CD8(+) T cells in the cancer-immunity cycle. *Immunity* 2023;56:2231-53.
32. Wang J, Lu Q, Chen X, et al. Targeting MHC-I inhibitory pathways for cancer immunotherapy. *Trends Immunol* 2024;45:177-87.
33. Huang Q, Wang F, Hao D, et al. Deciphering tumor-infiltrating dendritic cells in the single-cell era. *Exp Hematol Oncol* 2023;12:97.
34. Tai Y, Chen M, Wang F, et al. The role of dendritic cells in cancer immunity and therapeutic strategies. *Int Immunopharmacol* 2024;128:111548.
35. Syn NL, Teng MWL, Mok TSK, et al. De-novo and acquired resistance to immune checkpoint targeting. *Lancet Oncol* 2017;18:e731-41.
36. Qin S, Xu L, Yi M, et al. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. *Mol Cancer* 2019;18:155.
37. Azimnasab-Sorkhabi P, Soltani-Asl M, Kfoury Junior JR. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) as an undetermined tool in tumor cells. *Hum Cell* 2023;36:1225-32.
38. Ahmed MM, Gebriel MG, Morad EA, et al. Expression of Immune Checkpoint Regulators, Cytotoxic T-Lymphocyte Antigen-4, and Programmed Death-Ligand 1 in Epstein-Barr Virus-associated Nasopharyngeal Carcinoma. *Appl Immunohistochem Mol Morphol* 2021;29:401-8.
39. Abdelrahman DI, Elhasadi I, Anbaig A, et al. Immunohistochemical Expression of Immune Checkpoints; CTLA-4, LAG3, and TIM-3 in Cancer Cells and Tumor-infiltrating Lymphocytes (TILs) in Colorectal Carcinoma. *Appl Immunohistochem Mol Morphol* 2024;32:71-83.
40. Okuyama K, Naruse T, Yanamoto S. Tumor microenvironmental modification by the current target therapy for head and neck squamous cell carcinoma. *J Exp Clin Cancer Res* 2023;42:114.
41. Rosen RD, Sapra A. TNM Classification. Treasure Island (FL): StatPearls Publishing; 2025.

Cite this article as: Tang M, Chen Q, Xu X, Zhang Z, Han L, Wu H. PLCXD2 expression relates to the immune microenvironment and prognosis of head and neck squamous cell carcinoma: a retrospective cohort study. *Transl Cancer Res* 2025;14(10):6403-6413. doi: 10.21037/tcr-2025-880