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ORIGINAL ARTICLE

Prognostic Marker PARP11 in Head and Neck Squamous Cell Carcinoma and its Relationship with Immune Infiltration

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Abstract

Head and neck squamous cell carcinoma (HNSC) is one of the most common malignancies, and identification of HNSC biomarkers is critical. Polyadenosine 5'-diphosphate (ADP)-ribose polymerase (PARP) 11 is a member of the PARP family. The role of PARP11 in HNSC is unclear. The purpose of this article is to investigate the expression and prognostic value of PARP11 in HNSC patients, its potential biological functions, and its impact on the immune system. Gene expression and clinicopathological analysis, enrichment analysis, and immune infiltration analysis were based on The Cancer Genome Atlas (TCGA) data with additional bioinformatics analysis. Statistical analysis was performed using TIMER and ssGSEA to analyze the immune response to PARP11 expression in HNSCs. In addition, K-M survival analysis and data from HPA were used to validate the results. PARP11 played a vital role as an independent prognostic factor in HNSC patients. PARP11 expression correlated with age, stage, grade, and tumor status. GSEA found that PARP11 is closely related to cell adhesion, receptors, ribosomes, keratinization, and other functions. PARP11 expression was positively correlated with infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, dendritic cells, and neutrophils and was co-expressed with immune-related genes and immune checkpoints. The expression of PARP11 is increased in HNSC, and the high expression of PARP11 is associated with a poor prognosis. PARP11 may affect tumor development by regulating tumor-infiltrating cells in the tumor microenvironment (TME). PARP11 may be a potential target for immunotherapy.

Keywords: PARP11, HNSC, prognosis, tumor microenvironment, tumor immune cell infiltration

Introduction

HNSC[1] ranks sixth worldwide. HNSC is a malignant paranasal sinus, throat, and oral mucosa epithelial tumor[2]. Smoking, excessive alcohol use, and HPV infection are its key risk factors, and its etiology is varied and complex. More research is needed[3]. More than 600,000 new HNSC cases are diagnosed each year, and 380,000 die[4], representing for 5.7% of global cancer deaths[5]. Surgery, chemotherapy, and radiotherapy treat HNSC. Locally progressed, recurrent, and metastatic patients need better treatment[6]. Targeted and immunotherapy have been used to treat HNSC recently[7]. First FDA-approved HNSC-targeted treatment is cetuximab. Cetuximab improved survival by 2.7 months. Immunotherapy has grown because to targeted therapy's modest survival benefit[8]. To enhance HNSC diagnosis and prognosis, identify the main causes of tumor development and progression, which are immune-related[9, 10].

DNA damage repair mechanisms are promising cancer treatments. PARP, also known as ADP-ribosyltransferase, repairs DNA single-strand breaks by targeting proteins from NAD[+][11]. Unrepaired DNA double-strand breaks result from PARP inhibition[12]. At least 18 PARP family members have been identified[13]. PARP1's role in base excision repair (BER) single-strand break (SSB) DNA repair is the most researched[14]. Several cancers carry PARP1 mutations, and some point mutations are highly related with PARP1 drug resistance[15]. PARP2 regulates DNA repair, chromatin dynamics, and other activities[16]. PARP11 is a nuclear membrane-localized mono(ADP-ribosyl)transferase[16, 17]. PARP11 protein function is anticipated to be determined by the WWE domain and catalytic PARP domains. The WWE domain targets multiple E3-like and ADP ribosylation activities to receptors in various signaling cascades[18]. PARP11's distinct functional domains and function are unclear. PARP11, an interferon-stimulated gene that suppresses Zika virus (ZIKV) replication, may be crucial in the innate immune defense against ZIKV replication[11]. PARP11 is a new enzyme involved in sperm head shaping and nuclear envelope stability and reorganization during spermatogenesis. Idiopathic mammalian teratozoospermia factor[16] may be involved. PARP11 upregulates all glycosylated type I interferon receptors 1 (IFNAR1), including high-glucose, partial-glucose, and non/low-glucose[19]. PARP11's significance in HNSC, a frequent cancer, is unclear.

We examined PARP11 expression in human HNSC samples using TCGA data. PARP11 expression was correlated with clinical factors and HNSC patients' prognoses using R (version 3.6.3). We performed Gene Set Enrichment Analysis (GSEA), improved Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) investigations to better understand the biological processes connected with the PARP11 regulatory network that may underlie HNSC formation. We examined PARP11 and tumor-infiltrating immune cells (TIICs) using TIMER and ssGSEA. Finally, PARP11 and HNSC patients' prognoses were analyzed using K-M and HPA. The first PARP11-cancer study. Good models can predict prognosis, which can assist cancer patients choose treatment[8]. This study examined HNSC's independent risk factors and created a useful prognostic model.

2. Materials and Methods

2.1. Evidence from the TCGA database

This investigation used XXXXX, et al[20] methodologies. We collected immune cell infiltration, gene expression (workflow type: HTSeq-TPM), and clinical information (data type: Clinical Supplement) from the TCGA database of HNSCs[21]. We excluded samples due to insufficient age, TNM stage, OS time, distant and lymph node metastases, and local invasion information. RNA-Seq and clinical data were saved for research. Our study meets TCGA publishing requirements.

2.2. Gene Set Enrichment Analysis

Our GSEA analysis used TCGA's normalized RNA-Seq data[22]. Permutations default to 1000. We examined GO keywords and the KEGG pathway using GSEA to determine PARP11's biological functions. Gene Set Enrichment Analysis retrieved GO and KEGG gene sets. Enrichment findings must meet two criteria to be statistically significant. Nominal p-value <0.050, FDR <0.25.

2.3. Immune Infiltration Analysis

The TIMER correlation module (TIICs) examined PARP11 expression and tumor-invading immune cells. TIMER (https://cistrome.shinyapps.io/timer/) helps analyze immune infiltration in many malignancies[23]. TIMER used deconvolution, a novel statistical method, to estimate TIIC incidence from gene expression profiles[24]. TIMER estimates TIIC using 11093 TCGA samples from 32 cancer types. PARP11 expression in HNSCs and TIIC abundance across gene modules, including CD4+ T cells, dendritic cells, B cells, CD8+ T cells, B cells, neutrophils, and macrophages, were examined. TIMER graphed gene expression and tumor purity[25]. 24 immune cell types infiltrated tumors using ssGSEA. The Spearman correlation method was used to compare immune cell infiltration in high and low PARP11 expression groups. We examined the relationships between the 24 cells using a correlation heatmap, which shows the intensity of the interaction between every pair of immune

cells in a sample.

2.4. Immune Checkpoint Analysis

SIGLEC15, TIGIT, CTLA4, CD274, HAVCR2, LAG3, PDCD1, and PDCD1LG2 were connected with immunological checkpoints, hence their expression data was extracted. PARP11 expression and immunological checkpoints were examined. TIDE-predicted immune checkpoint blockade responses[26].

2.5. Comprehensive Analysis

Using TCGA data, we performed KM survival analysis (http://kmplot.com/analysis/) on PARP11 expression in HNSCs to compare OS and DSS[27]. KM survival curves assessed OS by clinicopathological factors. Immunohistochemical (IHC) staining photos from the Human Protein Atlas (HPA) (www.proteinatlas.org) were used to compare PARP11 protein expression in HNSC and head and neck normal tissues. HPA uses antibody profiling and protein expression profiles for 32 human tissues to locate proteins[28]. HPA also measures RNA.

2.6. Statistical Analysis

All statistical tests used R (version 3.6.3). Log2 transformation standardized gene expression levels. A two-sample t-test compared normal and cancerous samples. Kruskal-Wallis one-way ANOVA compared four-person groups. 95% CIs and HRs were calculated using univariate and multivariate Cox analysis. Univariate survival analysis compared clinical factors with survival. A multivariate Cox proportional hazards model examined PARP11 expression and other pathological and clinical factors (sex, age, grade, lymph node, distant metastasis, tumor status, and stage) on OS. Logistic regression examined clinical factors and PARP11 expression. Spearman's or Pearson's test can assess two variables' relationship. PARP11 expression was considered significant at 0.05.

3. Results

3.1. Survival Outcomes and Variable Analysis

We examined PARP11 mRNA levels in pan-cancer normal and tumor tissues using TCGA RNA-seq data. BRCA, CHOL, ESCA, GBM, HNSC, KICH, LIHC, LUAD, LUSC, PCPG, STAD, and THCA, except UCEC, had significantly varied PARP11 expressions. Seven cancers expressed PARP11 more than controls, whereas six expressed less. Figure 1 TCGA analyzed 502 HNSC tumors and 44 normal tissues for unpaired samples. PARP11 expression in standard and HNSC samples was 0.586 (0.401 - 0.769), P < 0.001 (Figure 2A). Pairwise comparisons showed a difference of 0.639 (0.403 - 0.876), P < 0.001 (Figure 2B). PARP11 expression was found in HNSC tumor tissues.

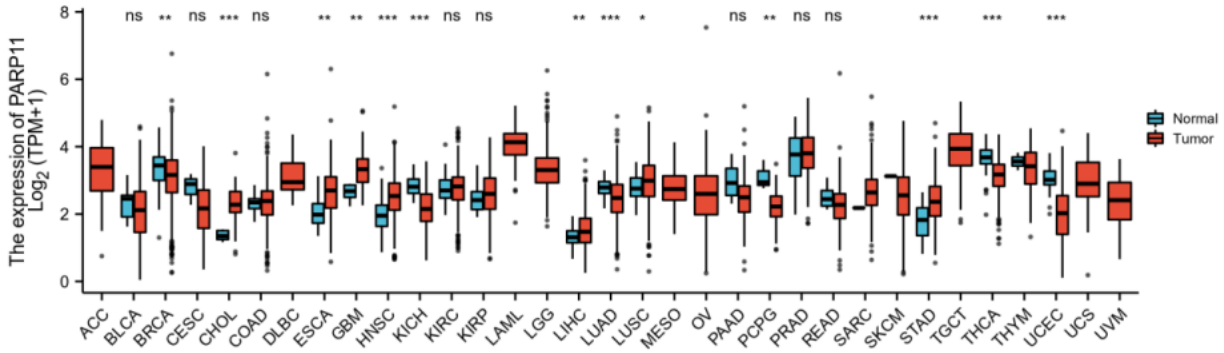

Figure 1. Expression of PARP11 in normal and pan-cancer tissues

3.2. Patient Characteristics

Table 1. Baseline data on PARP11 expression in HNSC patients, univariate and multivariate Cox proportional hazards analysis.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value

Age	501				
<=60	245（48.9）	Reference			
>60	256（51.1）	1.252 (0.956-1.639)	0.102	1.141 (0.738-1.763)	0.553
Gender	501				
Female	134（26.7）	Reference			
Male	367（73.3）	0.764 (0.574-1.018)	0.066	0.948 (0.596-1.508)	0.823
Histologic grade	482				
G1&G2	361（74.9）	Reference			
G3&G4	121（25.1）	0.939 (0.688-1.282)	0.692		
Clinical stage	487				
Stage I&Stage II	113（23.2）	Reference			
Stage III&Stage IV	374（76.8）	1.217 (0.878-1.688)	0.238		
T stage	486				
T1&T2	176（36.2）	Reference			
T3&T4	310（63.8）	1.245 (0.932-1.661)	0.137	2.036 (1.178-3.517)	**0.011**
N stage	479				
N0	238（49.69）	Reference			
N1&N2&N3	241（50.31）	1.263 (0.964-1.653)	0.090	1.402 (0.875-2.247)	0.160
M stage	476				
M0	471（98.95）	Reference			
M1	5（1.05）	4.745 (1.748-12.883)	**0.002**	4.506 (0.551-36.853)	0.160
Smoker	491				
Yes	380（77.4）	Reference			
No	111（22.6）	0.918 (0.656-1.285)	0.618		
Alcohol history	490				
Yes	332（67.8）	Reference			
No	158（32.2）	1.051 (0.790-1.397)	0.733		
Lymphovascular invasion	340				
Yes	122（35.9）	Reference			
No	218（64.1）	0.589 (0.419-0.826)	**0.002**	0.582 (0.370-0.915)	**0.019**
Lymphnode neck dissection	498				
Yes	408（81.9）	Reference			
No	90（18.1）	1.368 (0.984-1.901)	0.062	1.505 (0.624-3.631)	0.363
Primary therapy outcome	417				

SD&PD	47 (11.3)	Reference			
CR&PR	370 (88.7)	0.181 (0.122-0.269)	**<0.001**	0.209 (0.124-0.352)	**<0.001**
Radiation therapy	440				
Yes	287 (65.2)	Reference			
No	153 (34.8)	1.631 (1.203-2.212)	**0.002**	2.157 (1.310-3.552)	**0.003**
PARP11 (Low vs. High)	501	1.373 (1.050-1.795)	**0.021**	1.853 (1.210-2.839)	**0.005**

Table 1 shows TCGA baseline data from March 2022, which included 502 primary cancers with clinical and gene expression data. 48.9% of patients were under 60, while 51.1% were over 60. 134 women and 367 men. Histologic grade was G1&G2 in 361 (74.90%) and G3&G4 in 121 (25.10%). Stage I&II, 113 cases (23.20%), and Stage III&IV, 374 cases (76.80%), comprised the clinical stage. 176 (36.21%) of 486 T-stage patients were T1&T2, and 310 (63.79%) were T3&T4. 238 N0 instances (49.69%) and 241 N1&N2&N3 cases (50.31%). M staging showed 471 M0 (98.95%) and 5 M1 (1.05%) instances. 332 (67.76%) drank and 380 (77.39%) smoked. 122 instances (35.88%) had lymphovascular invasion, while 408 cases (81.93%) had lymph node neck dissection. CR&PR had 370 cases (88.73%) and SD&PD 47 (11.27%). 287 patients (65.23%) underwent radiation. 251 instances expressed PARP11 lowly and 250 highly. As reported in Table 1, we utilized Cox analysis to examine PARP11 expression and OS and other multivariate features of HNSC patients (Figure 2D). Finally, in our multivariate analysis, T stage, lymphovascular invasion, primary therapeutic result, radiation therapy, and PARP11 were independent risk factors for HNSC. PARP11 expression predicted survival with an AUC of 0.753 (Figure 2C).

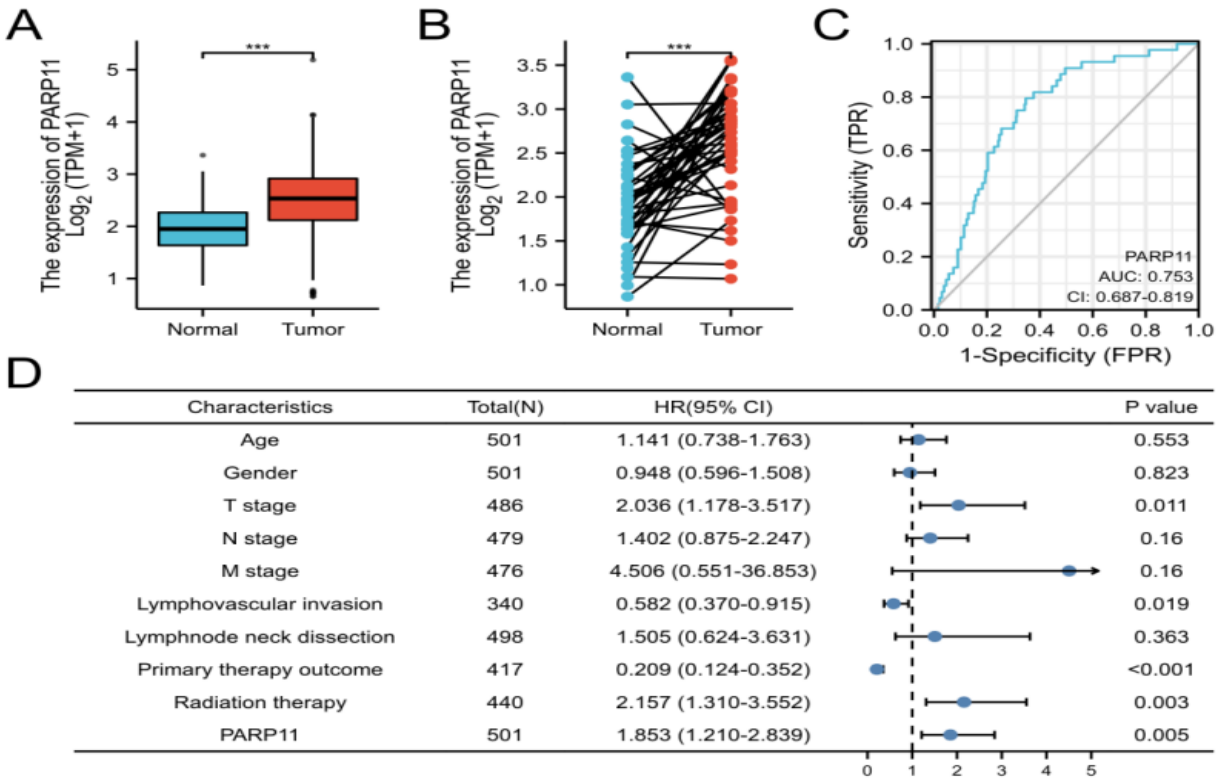

Figure 2. PARP11 expression in HNSC tissues. (A) PARP11 expression in normal and tumor tissues. (B) PARP11 expression in paired tissues. (C) ROC curve of PARP11. (D) PARP11 expression and other Multivariate Cox analysis forest plot of clinicopathological variables.

3.3. Relationship between PARP11 Expression and Clinicopathology

PARP11 expression levels and HNSC clinicopathological characteristics were examined. 502 HNSC samples having PARP11 expression data from diverse clinical features are in the TCGA. High PARP11 expression was associated with worse OS (Figure 3), expressed in the following factors: Age (P=0.021), Female (P=0.002), Histologic grade (P=0.019), T stage (P=0.034), N stage (P=0.027), M stage (P=0.041), Clinical stage (P=0.031), Smoker (P=0.021), Alcohol history (P=0.015), Radiation therapy (P=0.024), Lymphovascular invasion (P=0.025), Lymph node neck dissection (P=0.017), Primary therapy outcome (P=0.008). Male was uncorrelated (P = 0.412). Increased PARP11 expression was substantially linked with Histologic grade (Figure 4A) and Clinical stage (Figure 4B). Univariate logistic regression demonstrated that PARP11 expression was linked with specific clinical features of HNSC patients (Table 2). PARP11 expression was substantially linked with Histologic grade (G3&G4 vs. G1&G2, OR=1.838, P-value = 0.004) and Clinical stage (Stage III&IV vs. Stage I&II, OR=0.614, P=0.025). These findings show that HNSC patients with high PARP11 expression are more likely to have malignancies of higher grade and clinical stage.

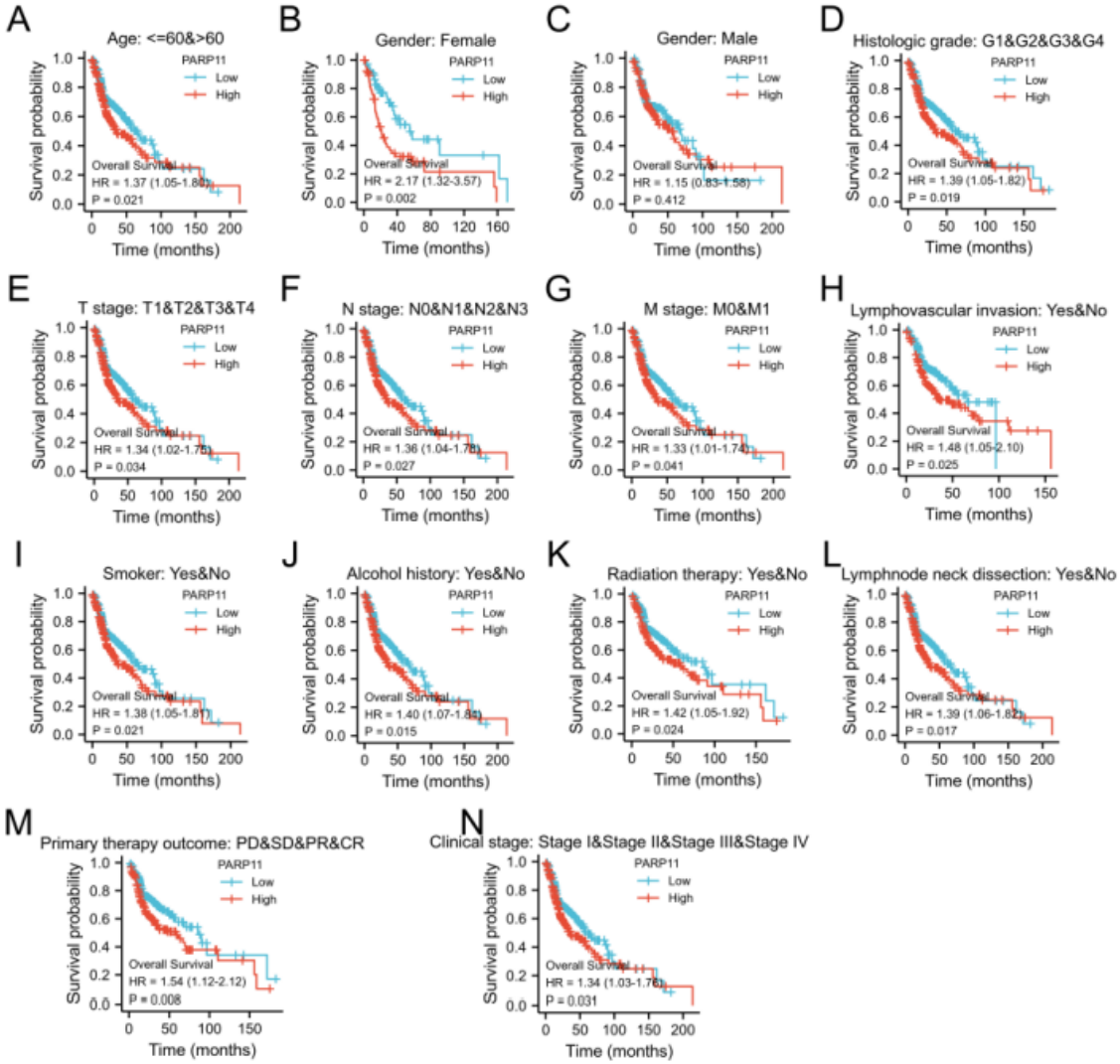

Figure 3. Kaplan-Meier estimate of overall survival of patients by (A) Age, (B) Female, (C) Male, (D) Histologic grade, (E) T stage, (F) N stage, (G) M stage, (H) Lymphovascular invasion, (I) Smoker, (J) Alcohol history, (K)Radiation therapy, (L) Lymphnode neck dissection, (M) Primary therapy outcome, (N) Clinical stage.

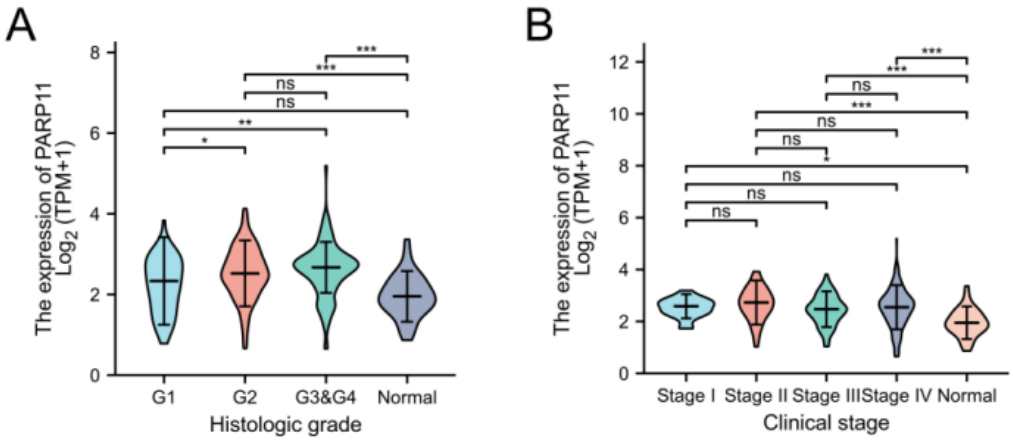

Figure 4. Expression of PARP11 correlated significantly with histological grade (A), and clinical stage (B).

Table 2. Logistic regression analysis of the correlation between PARP11 expression and clinicopathological factors.

Characteristics	Total(N)	Odds Ratio(OR)	P value
Age (>60 vs. <=60)	501	0.819 (0.576-1.162)	0.264
Histologic grade (G3&G4 vs. G1&G2)	483	1.838 (1.212-2.811)	0.004
Clinical stage (Stage III&Stage IV vs. Stage I&Stage II)	488	0.614 (0.400-0.938)	0.025
T stage (T3&T4 vs. T1&T2)	487	0.711 (0.489-1.029)	0.071
N stage (N1&N2&N3 vs. N0)	480	0.791 (0.552-1.132)	0.201
M stage (M1 vs. M0)	477	0.628 (0.082-3.824)	0.612

3.4. GSEA investigation of PARP11

GO word and KEGG pathway analysis investigated PARP11's biological roles. GSEA showed that high PARP11 samples had significantly distinct KEGG pathway and GO word enrichment (FDR < 0.25, p-value < 0.050) (Table 3).

Table 3. Signaling pathways most significantly correlated with PARP11 expression based on their normalized enrichment score (NES) and p-value.

A	KEGG NAME	NES	p values	q values
positive	CELL_ADHESION_MOLECULES_CAMS	3.22639	0.00362	0.01049
	ECM_RECEPTOR_INTERACTION	3.06647	0.00346	0.01043
	FOCAL_ADHESION	2.89734	0.00418	0.01143
	HEMATOPOIETIC_CELL_LINEAGE	2.86346	0.00343	0.01043
	INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	2.78941	0.00307	0.01043
negative	RIBOSOME	3.40806	0.00140	0.01043
	OXIDATIVE_PHOSPHORYLATION	2.89638	0.00139	0.01043
	PARKINSONS_DISEASE	2.58978	0.00141	0.01043
	ALZHEIMERS_DISEASE	2.27721	0.00136	0.01043
	HUNTINGTONS_DISEASE	2.18111	0.00134	0.01043
B	GO NAME	NES	P value	q values
positive	T_CELL_RECEPTOR_COMPLEX	3.83603	0.00362	0.01128
	EXTRACELLULAR_MATRIX_STRUCTURAL_CONSTITUENT	3.43940	0.00392	0.01128
	PLASMA_MEMBRANE_SIGNALING_RECEPTOR_	3.43074	0.00503	0.01200

	COMPLEX			
	RECEPTOR_COMPLEX	3.38250	0.00704	0.01425
	INTRINSIC_COMPONENT_OF_SYNAPTIC_ MEMBRANE	3.23070	0.00412	0.01134
negative	KERATINIZATION	-3.43242	0.00127	0.01128
	CORNIFICATION	-3.40068	0.00140	0.01128
	COTRANSLATIONAL_PROTEIN_TARGETING_TO _MEMBRANE	-3.37151	0.00138	0.01128
	KERATINOCYTE_DIFFERENTIATION	-3.34879	0.00124	0.01128
	CYTOSOLIC_RIBOSOME	-3.26650	0.00140	0.01128

Normalized enrichment scores (NES) determined the most enriched signaling pathways. KEGG pathway analysis identified five pathways with the most positive link with PARP11 expression (Figure 5A): cell adhesion molecules cams, ECM receptor interaction, focal adhesion, hematopoietic cell lineage, and intestinal immune network for IgA synthesis. Ribosome, oxidative phosphorylation, Parkinson's, Alzheimer's, and Huntington's diseases had the highest negative relationships (Figure 5B). Figure 5C shows five GO classes positively linked with increased PARP11 levels:T cell receptor complex, extracellular matrix structural constituent, plasma membrane signaling receptor complex, receptor complex, and synaptic membrane intrinsic component.

Keratinization, cornification, cotranslational protein targeting membrane, keratinocyte differentiation, and the cytosolic ribosome had the highest negative connections in GO analysis (Figure 5D). PARP11 expression affects HNSC illnesses through pathways such cell adhesion, receptors, nervous system abnormalities, ribosomes, and keratinization.

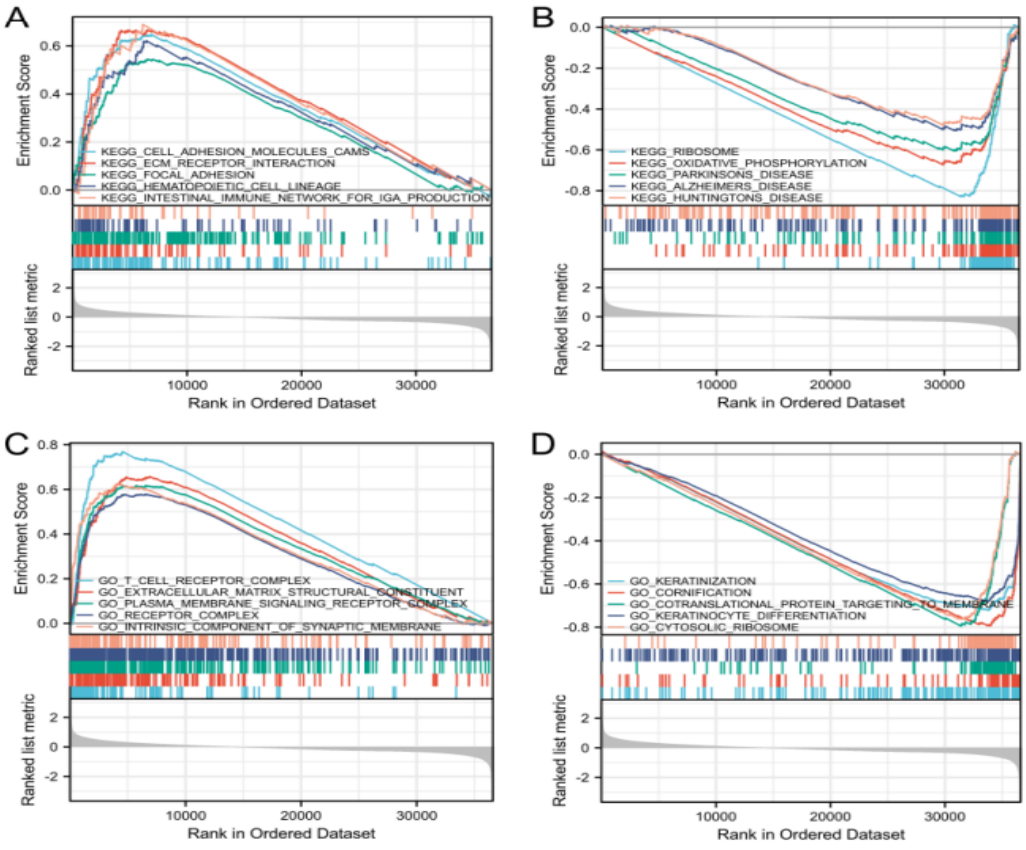

Figure 5. KEGG pathway showed five positively correlated groups (A), and five negatively correlated groups (B), GO term analysis revealed five positively correlated groups (C), and five negatively

correlated groups (D).

3.5. The Relationship between PARP11 Expression and Tumor-Infiltrating Immune Cells

Independent tumor-infiltrating lymphocytes predict OS and sentinel lymph node status[29]. Thus, we examined PARP11 expression and HNSC immune infiltration using TIMER. As shown in Figure 6A, PARP11 expression was not significantly correlated with tumor purity (r = -0.008, P = 8.54e-01), with B cells (r = 0.123, p-value = 7.44e-03), CD8 + T cells (r=0.104, p-value=2.39e-02), CD4+ T cells (r=0.45, p-value=2.56e-25), macrophages (r=0.297, p-value=2.61e- 11), neutrophils (r = 0.369, p-value = 7.05e-17) and dendritic cells (r = 0.394, p-value = 2.57e-19) were significantly positively correlated. PARP11 expression levels are related with poorer prognosis and greater immune infiltration in HNSC patients. PARP11 enhances HNSC immune infiltration..

Next, the relationship between PARP11 expression and 24 different immune cell types was assessed in HNSCs. PARP11 expression is closely related to Th2 cells, T helper cells, NK cells, Tcm, Eosinophils, TFH, Macrophages, Treg, Tem,aDC, Th1 cells, T cells, iDC, CD8 + T cells were closely correlated. (Figure 6B) Further studies revealed significant differences in PARP11 expression levels among infiltrating immune cells, including T cells, aDCs, CD8+ T cells, Eosinophils, iDCs, Macrophages, NK cells, T helper cells, Tcm, Tem, TFH, Th1 cells, Th2 cells, TReg were affected by PARP11 expression. Compared with the PARP11 low expression group, increased in the PARP11 high expression group, including T cells, aDC, CD8 T, Eosinophils, iDC, Macrophages, NK cells, T helper cells, Tcm, Tem, TFH, Th1 cells, Th2 cells, Treg, the difference was statistically significant ($P < 0.05$). But B cells, Cytotoxic cells, DC, Mast cells, Neutrophils, and NK CD56dim cells were not significantly different ($P > 0.05$). Compared with the PARP11 low expression group, the PARP11 high expression group decreased, including NK CD56bright cells, pDC, Tgd, and Th17 cells, and the difference was not statistically significant ($P > 0.05$). (Figure 6C) We also assessed possible correlations between 24 immune cells. (Figure 6D) The resulting heatmap indicated that the ratios of different tumor-infiltrating immune cell subsets were strong to moderately correlated.

In PARP11 low and high HNSCs, immunological checkpoints TIGIT, SIGLEC15, CTLA4, HAVCR2, LAG3, CD274, PDCD1, and PDCD1LG2 were examined. The PARP11 high expression group of HNSCs had significantly higher immune checkpoint expression (Figure 6E). We found that immune checkpoint blockage worked better in HNSCs with increased PARP11 expression (Figure 6F). PARP11 was positively co-expressed with various immunological checkpoints (Table 4), suggesting it may be an immunotherapy target.

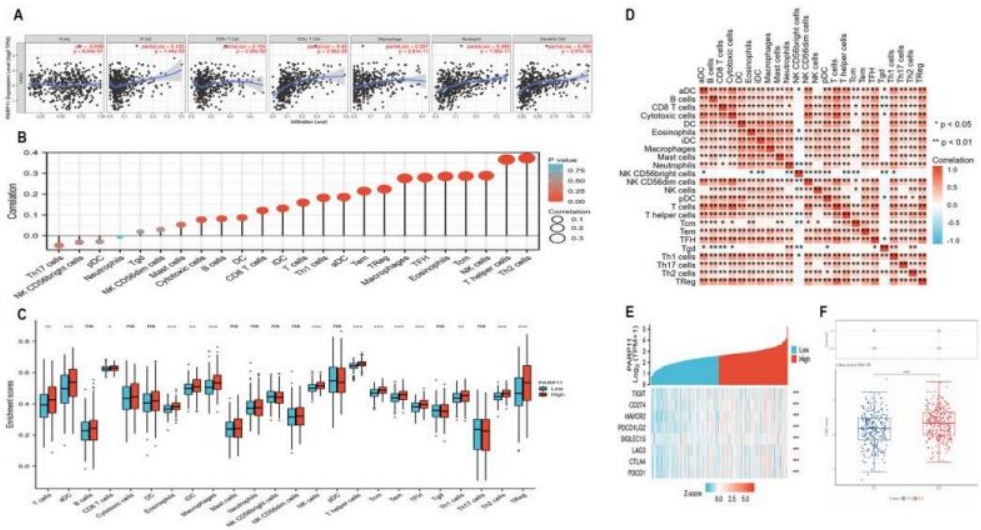

Figure 6. The correlation between PARP11 expression and immune cell infiltration in tumor

microenvironment and the expression of immune checkpoints in HNSC. (A) The relationship between PARP11 expression and six types of immune cell infiltration levels. (B) PARP11 expression and 24 types of immune cells Relationships between immune cells. (C) Change ratio of 24 immune cell subtypes in high and low PARP11 expression groups in tumor samples. (D) Heat map of 24 immune infiltrating cells in tumor samples. (E) Low PARP11 expression Differential expression of immune checkpoints with high and high expression. (F) Differential responses of low (G1) and high (G2) PARP11 expression to immune checkpoint blockade.

Table 4. Correlation of PARP11 with immune checkpoints in HNSC.

Genes	Cor	p-value
TIGIT	0.329	<0.001
CD274	0.262	<0.001
HAVCR2	0.381	<0.001
PDCD1LG2	0.344	<0.001
SIGLEC15	0.183	<0.001
LAG3	0.226	<0.001
CTLA4	0.308	<0.001
PDCD1	0.262	<0.001

3.6. Data verification

The KM survival plot also showed that the group with high PARP11 expression had a low OS (P-value=0.021, Figure 7A) and DSS (P-value=0.024, Figure 7B). We projected the nomogram to predict HNSC OS based on independent risk factors (Figure 7D) and demonstrated strong predictive value in the calibration curve (Figure 7C). HPA immunohistochemical examination showed that tumor tissue expressed PARP11 more than non-tumor tissue (Figure 7E and 7F).

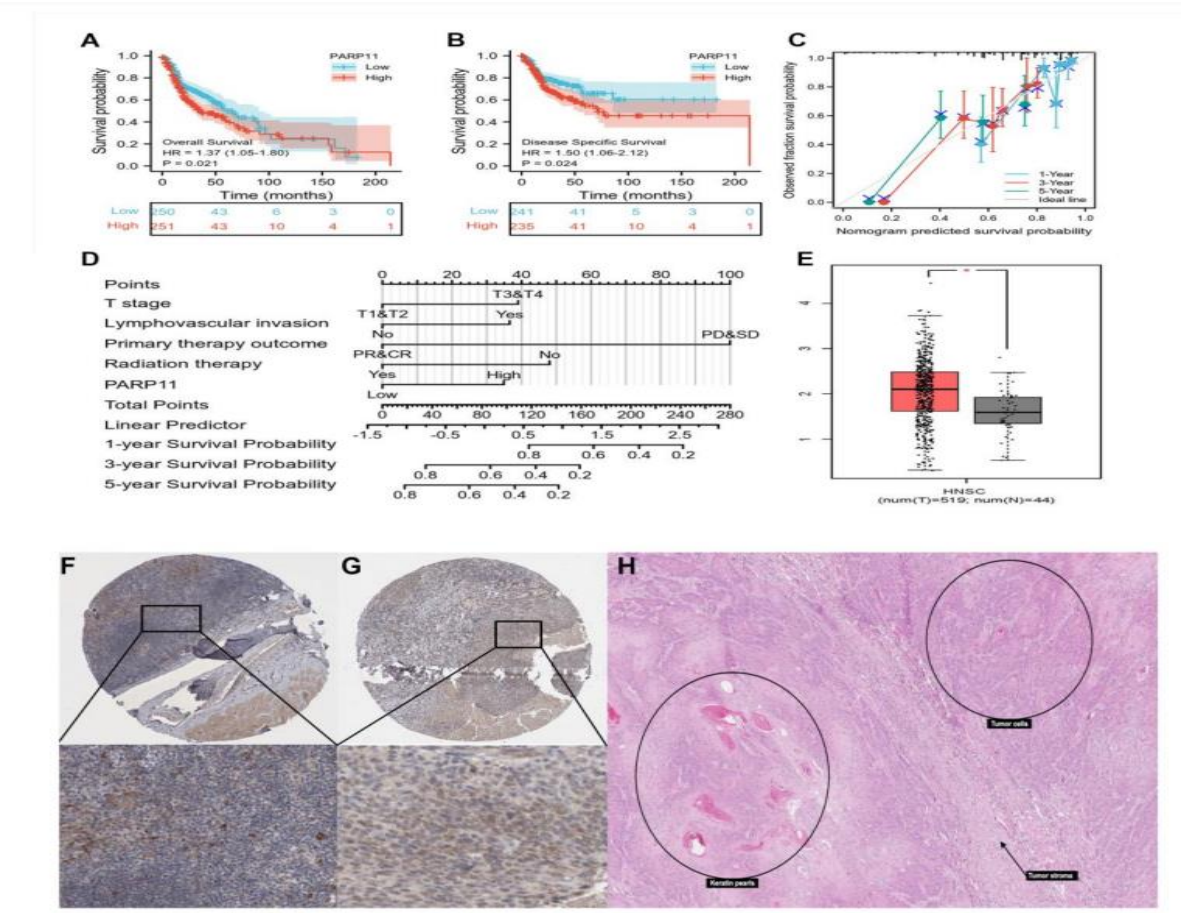

Figure 7. Prognostic analysis of PARP11 expression. Compared with patients with low PARP11 expression, patients with high PARP11 expression had poorer prognosis, including overall survival (OS) (A), disease-specific survival (DSS) (B)) (two log-rank P < 0.05). (C) Calibration plot showing the predictive performance of the model constructed using multivariate Cox regression analysis. (D) Multivariate analysis nomogram of clinical features based on PARP11 expression. (E) Expression levels of PARP11 mRNA in normal and HNSC tissues obtained from GEPIA. Head and neck normal tissues were observed using HPA immunohistochemistry (F), PARP11 protein expression in HNSC tissues (G), HNSC hematoxylin- eosin staining (H).

Discussion

With rising global population and life expectancy, HNSC has become one of the most frequent cancers[30]. Due to the high percentage of advanced disease diagnosis, HNSC mortality has not decreased despite advances in diagnosis and treatment[31]. HNSC patients can survive 80-90% if diagnosed and treated early[30]. HNSC distant metastases are rare compared to other malignancies, however survival beyond 24 months is less than 26%[32]. Cancer cells develop by metabolic, growth, adhesion, and proliferation changes[33]. HNSC, which are engaged in several cell cycle phases, have been diagnosed with several biomarkers recently. MMPs, which promote tumor invasion and metastasis, dramatically increase HNSC patients' serum and are useful biomarkers for diagnosis[10]. HNSC CCT3 mRNA and protein expression was considerably increased and linked with several clinicopathological characteristics. Parameter linked. CCT3 overexpression was related with worse HNSCC survival. HNSC's metastatic ability and CCT3 may be therapeutic targets[34]. HNSCs upregulate FOXD1 mRNA and protein, which may be prognostic indicators. The tumor microenvironment (TME) and immune cell invasion depend on FOXD1 expression[35]. HNSC with elevated CXCR5 have a positive prognosis[36].

Poly(ADP-ribose) polymerase (PARP) is an abundant ribozyme involved in DNA repair, gene expression, cell cycle control, and energy metabolism[37, 38]. PARP1 and PARP2 also play roles in transcription, apoptosis, and immunological function[39]. Thus, PARP

expression affects carcinogenesis and progression. HNSC PARP family research is scarce. PARP inhibitors may be chemical potentiators or radiosensitizers, according to extensive clinical data[40]. Again, reduced DNA damage response[41] and dependency on cellular replication[42] may be responsible.

This work is the first to examine PARP11 as a biomarker for HNSC prognosis using TCGA data. HNSC cancers expressed more PARP11 than head and neck normal tissues. HNSC patients with elevated PARP11 expression had poor survival. This study also found PARP11, T stage, lymphovascular invasion, primary therapy result, and radiation therapy as independent risk variables. Valid HNSC predictive nomogram for 1-, 3-, and 5-year OS. HNSC patients with strong PARP11 expression had higher grades and stages. PARP11 overexpression may impact HNSC progression tumorigenesis and immunology.

In this study, up-regulated PARP11 increases HNSC cell adhesion and receptor activities and inhibits ribosome and keratinization. Overexpression of PARP11 in HNSC patients may promote cell adhesion, plasma membrane receptor-related effects, and metastasis by decreasing ribosomal activity and keratinization. More research is needed to determine PARP11's roles in HNSCs. Our findings help explain why PARP11 overexpression in HNSCs is particularly harmful. Targeted therapies like PARP inhibitors are interesting new treatments for ovarian, breast, pancreatic, and prostate cancers. Olaparib, rucaparib, niraparib, and talazoparib were approved by the FDA for advanced ovarian or breast cancer in 2014, 2016, 2017, and 2018[13, 39]. HNSC PARP inhibitor research is nonexistent. Our findings will aid PARP inhibitor development and use in HNSC.

Tumor growth depends on the immunological microenvironment[43]. TME profiles can predict tumor prognosis and tumor cell response to immunotherapy[44]. HNSC progression is linked to immunological alterations in the tumor microenvironment[29, 45, 46]. Various mechanisms. This single-cell database analysis is the first to identify PARP11 as an immune-related prognostic marker in the HNSC tumor microenvironment. We used the TIMER database to reveal the link between PARP11 expression and the level of immune infiltration in HNSCs. As shown in Figure 6A, PARP11 was significantly positively correlated with the infiltration of B Cell, CD8+ T Cell, CD4+ T Cell, Macrophage, Neutrophil, and Dendritic Cell. This study further investigated the differences in the infiltration of 24 types of immune cells between HNSC patients with high and low PARP11 expression. The results based on ssGSEA analysis showed that the expression level of PARP11 was closely related to Th2 cells, T helper cells, NK cells, Tcm, Eosinophils, TFH, Macrophages, Treg, Tem, aDC, Th1 cells, T cells, iDC, CD8 + T cells, DC, The infiltration levels of B cells, Cytotoxic cells and Mast cells were significantly positively correlated, and significantly negatively correlated with Th17 cells. These relationships suggest an essential role for PARP11 in regulating HNSC tumor immunology. In Figure 6C, the results showed significant differences in the PARP11 expression level in the immune cells, including T cells, aDC, CD8+T cells, Eosinophils, iDC, Macrophages, NK cells, T helper cells, Tcm, Tem, TFH, Th1 cells, Th2 cells, TReg. These findings collectively suggest that PARP11 likely plays a vital role in regulating the immune function of HNSCs. However, we still need to conduct controlled and multicenter clinical trials to understand the relationship between PARP11 and immune cells in vivo.

PARP11 was positively co-expressed with immunological checkpoints TIGIT, CD274, PDCD1, HAVCR2, PDCD1LG2, SIGLEC15, LAG3, and CTLA4. HNSCs with high PARP11 expression expressed more immune checkpoints and responded better to immune checkpoint blockage than those with low PARP11. These findings suggest that HNSC patients with high PARP11 expression may benefit from immune checkpoint blockade therapy and that PARP11 may be a target for immunotherapy.

This study has limitations. First, our study data is based on online platform databases that are regularly updated and enlarged, which may alter the results. Second, our study lacked consequences and treatment details. Third, in vivo and in vitro tests did not confirm PARP11's function in HNSC immunity and its molecular mechanism. Thus, in future investigations, we will focus on patient baseline data and conduct more trials to confirm the projected findings.

Conclusion

In summary, this is the first report to identify PARP11 as a novel prognostic marker in HNSC and to suggest that it is associated with immune function. With a better understanding of its functional scope, PARP11 could serve as an effective tool for diagnosing and treating HNSC and may help make biomarker therapy a promising option for treating HNSC. However, the mechanism by which PARP11 promotes tumor progression and metastasis in HNSCs still needs to be further elucidated.

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Conflict of Interest Statement

All authors declared that there was no conflict of interest.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

All authors have agreed to the publication.

Informed Cnsent

Informed consent was obtained from all individuals included in this study.

Authors’ Contributions

Hesen Huang: Writing - original draft, Investigation, Conceptualization, Visualization, Formal analysis. Yu Du: Writing – original draft, Investigation, Conceptualization.: Writing - original draft, Conceptualization. Kaiqin Chen: Resources, Writing - review & editing, Project administration, Supervision.

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