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PBK Expression Is Associated With Prognosis of Patients With Oral Squamous Cell Carcinoma Treated With Radiotherapy: A Retrospective Study

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Abstract. *Background/Aim: To investigate the impact of PDZ-binding kinase (PBK) on the clinical outcome of patients with oral squamous cell carcinoma (OSCC) who received radiotherapy. Patients and Methods: PBK immunoreactivity of cancer specimens obtained from 179 patients with primary OSCC was analyzed by immunohistochemistry. Results: High PBK expression in tumor cells tended to be associated with advanced N-stage. The 5-year survival rate was greater for patients with high total PBK expression than in those with low PBK expression. After adjustment, high PBK remained associated with a favorable outcome. In subgroups according to tumor stage, the prognostic role was significant in patients with stage III/IV rather than those with stage I/II disease. Conclusion: We suggest that PBK expression should be used as an independent prognostic marker for patients with OSCC treated with radiotherapy, especially for those with advanced-stage disease.*

Oral squamous cell carcinomas (OSCCs) are the most common among all head and neck squamous cell cancers (1). In 2020, cancer of the lip and oral cavity accounted for more than 377,713 cases and 177,757 deaths worldwide (2). Individuals from developing countries where there is higher number of risk factors, such as smoking, betel nut chewing, and alcohol consumption, are at an increased risk for developing OSCC. In Taiwan, OSCC is the fourth common cancer type (approximate incidence rate of 29 per 100,000 population and mortality of 32 per 100,000 population) and the second cancer type with the fastest increasing incidence (3). The Surveillance, Epidemiology, and End Results Cancer Statistics Review reported that the 5-year relative survival rate patients with locally advanced oral cavity and oropharyngeal cancer is 54.7% whereas it is 82.5% for those with early-stage disease (4).

Many efforts have been made to identify biomarkers or specific genes that might provide useful information for clinical patient management. The complex pathogenesis of oral cancer is driven by DNA-repair genes, tumor-suppressor genes, and well-recognized factors, such as alcohol, betel nut chewing, and viral infection (5, 6). PDZ-binding kinase (PBK), also known as lymphokine-activated killer T-cell-originated protein kinase, is a mitogen-activated protein kinase kinase-like serine/threonine kinase that is involved in cell-cycle regulation *via* a cyclin B1-dependent manner, and in mitotic progression (7-9). PBK is found in proliferative tissues, such as testis, fetal, and neuronal stem cells; studies have found PBK overexpression in various malignancies, such as leukemia, Burkitt's lymphoma, breast cancer, and lung cancer (10-13). PBK is up-regulated in tumors; however, reports on the clinical significance of PBK are lacking. Our previous research showed the unfavorable

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Key Words: PBK, PDZ binding kinase, prognosis, oral cancer, radiotherapy.

Table I. *Relationships between PDZ-binding kinase (PBK) expression and clinical parameters in 179 patients with oral squamous cell carcinoma treated with radiotherapy.*

Parameter		Cases, n (%)	PBK expression		*p*-Value
			Low	High	
Age, years	Mean±SD	179	55.1±10.7	58.6±11.7	0.227
Gender, n (%)	Female	27 (15.1)	21 (77.8)	6 (22.2)	0.559
	Male	152 (84.9)	110 (72.4)	42 (27.6)	
Smoking, n (%)	No	101 (56.4)	69 (68.3)	32 (31.7)	0.094
	Yes	78 (43.6)	62 (79.5)	16 (20.5)	
Betel quid chewing, n (%)	No	141 (78.8)	101 (71.6)	40 (28.4)	0.366
	Yes	38 (21.2)	30 (78.9)	8 (21.1)	
Alcohol consumption, n (%)	No	104 (58.1)	80 (76.9)	24 (23.1)	0.184
	Yes	75 (41.9)	51 (68.0)	24 (32.0)	
Differentiation, n (%)	Well	16 (8.9)	14 (87.5)	2 (12.5)	0.383
	Moderate	157 (87.7)	113 (72.0)	44 (28.0)	
	Poor	6 (3.4)	4 (66.7)	2 (33.3)	
Stage, n (%)	I+II	47 (26.3)	37 (78.7)	10 (21.3)	0.318
	III+IV	132 (73.7)	94 (71.2)	38 (28.8)	
T-Stage	1+2+3	108 (60.3)	79 (73.1)	29 (26.9)	0.989
	4	71 (39.7)	52 (73.2)	19 (26.8)	
N-Stage	0	82 (45.8)	65 (79.3)	17 (20.7)	0.091
	1+2+3	97 (54.2)	66 (68.0)	31 (26.8)	

PBK: PDZ-binding kinase.

clinical prognosis of patients with OSCC with low PBK expression (14). Primary surgery and definitive radiation therapy are treatment options for patients with early- and advanced-stage OSCC (15). Combined modalities are generally recommended for approximately 60% of patients with locally or regionally advanced OSCC. In this study, we further evaluated the clinical prognostic role of PBK in patients with OSCC treated with radiotherapy.

Patients and Methods

Study subjects and ethics statement. In this retrospective study, tumor tissues from a tissue bank of 179 patients with OSCC diagnosed between 2000 and 2007 at Changhua Christian Hospital were screened for this survey. Patients with history of other malignancies or with missing clinical data were excluded. A total of 95 patients died during this survey. Cancers were staged according to the seventh edition of the American Joint Committee on Cancer Staging Manual (16). Clinical data were obtained from medical records and from the cancer registry. The survival time was defined as the duration between pathologically proven disease and the end of collection of available data or death. The Institutional Review Board and the Ethics Committee of the Changhua Christian Hospital, Changhua, Taiwan (Institutional Review Board No. 121008) gave their approval as well as waived the consent for this study.

Immunohistochemistry staining of PBK expression in tumors. Immunohistochemistry staining was performed at the Department of Surgical Pathology, Changhua Christian Hospital, as previously described (17, 18). Tissue microarray sections (4 μm) of formalin-fixed, paraffin-embedded primary oral tumors were analyzed. The sections were placed on coated slides, washed with xylene to remove the paraffin, and rehydrated through serial dilutions of alcohol, followed by washing with a solution of phosphate-buffered saline (PBS, pH 7.2). Endogenous peroxidase activity was blocked with 3% H_2O_2. Antigen retrieval was performed by boiling in citrate buffer (10 mM) for 20 min. The antibody used was anti-human PBK (PBK antibody, sc-136026, 1:150 dilution; Santa Cruz Biotechnology, Dallas, TX, USA). After incubation with primary antibody for 20 min at room temperature and thorough washing (three times with PBS), the slides were incubated with a horseradish peroxidase–Fab polymer conjugate for another 30 min. The sites of peroxidase activity were visualized using 3,39-diamino-benzidine tetrahydrochloride as the substrate and hematoxylin as the counterstain. PBS was used instead of primary antibodies as a negative control. Immunoreactivity was analyzed by pathologists independently according to a previously described scoring system (17, 19). Immunostaining scores were defined as the cell staining intensity score (range from 0 to 3, representing no staining to strong staining) multiplied by the percentage of stained cells (0-100%), leading to scores from 0 to 300. The cut-off value for positivity according to the PBK expression score was 50 which was the third quartile of this study population.

Statistical analysis. Student *t*-test and chi-square test were applied for continuous or discrete data analysis. Associations between PBK score and prognosis were estimated using the Kaplan–Meier method with log-rank test. Analysis of time-varying covariate showed no statistical significance. Potential confounders were adjusted for by Cox regression models, with PBK score fitted as indicator variable. In the multivariate analysis, gender and stage were adjusted for. All statistical analyses were conducted using the SPSS statistical

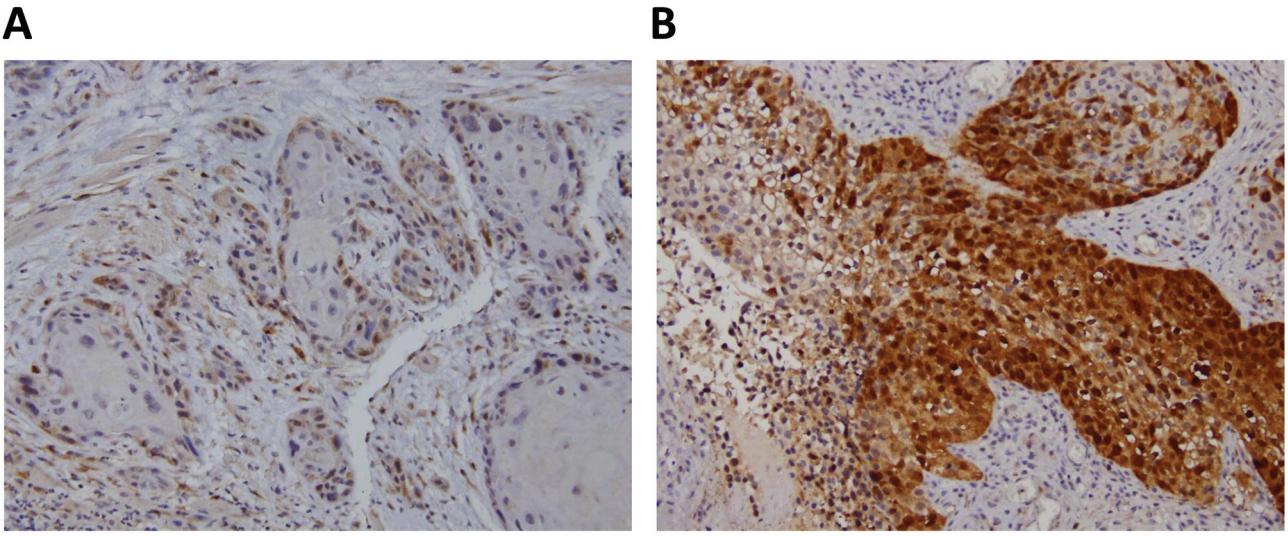

Figure 1. *Representative immunostaining showing low (A) and high (B) PDZ-binding kinase expression in oral squamous cell carcinoma specimens. Original magnification, ×100.*

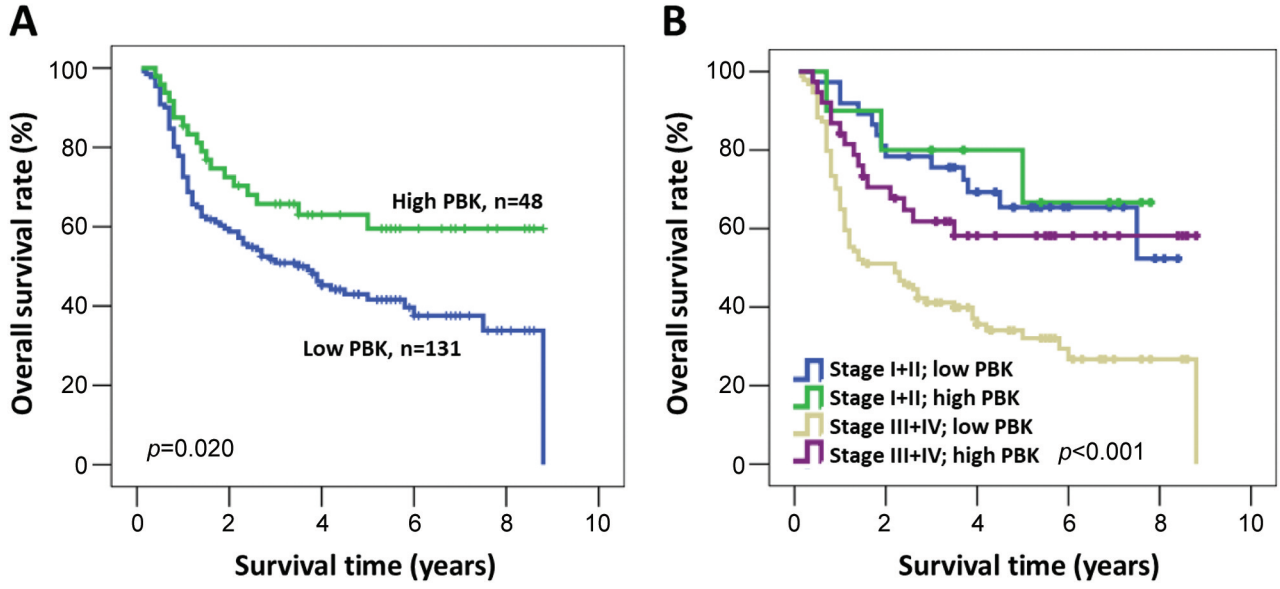

Figure 2. *Kaplan–Meier actuarial analysis of overall survival of patients with oral squamous cell carcinoma according to PDZ-binding kinase (PBK) expression (A) and PBK expression by stage (B).*

software program (version 15.0) (SPSS, Inc., Chicago, IL, USA). All statistical tests were two-sided, and *p*-values of less than 0.050 indicated statistical significance.

Results

Patient characteristics and the relationship between PBK expression and clinical parameters. We evaluated 179 patients with OSCC treated with radiotherapy, and their clinicopathological characteristics are listed in Table I. The representative immunostaining of PBK in OSCC specimens are shown in Figure 1. The mean age±standard deviation was 56.0±11.0 years, and the gender ratio (female:male) was 0.17:1.00. Notably, in this study population, the frequencies of patients with risk factors for developing oral malignancy (namely smoking, betel quid chewing, and alcohol consumption) were relatively low (43.6%, 21.2%, and 41.9%,

Table II. *Univariate analysis of the influence of various parameters on the overall survival of patients with oral squamous cell carcinoma.*

		Overall survival			
Parameter	Category	5-Year (%)	HR	95% CI	*p*-Value
Age	≥57 *vs.* <57 Years	46.1 *vs.* 46.4	0.973	0.647-1.462	0.894
Gender	Male *vs.* female	43.1 *vs.* 65.7	2.019	1.013-4.026	**0.046**
Smoking	Yes *vs.* no	50.1 *vs.* 43.3	0.826	0.548-1.246	0.362
Betel quid chewing	Yes *vs.* no	57.2 *vs.* 43.1	0.618	0.356-1.074	0.088
Alcohol consumption	Yes *vs.* no	46.6 *vs.* 45.8	0.996	0.662-1.498	0.983
Stage	III+IV *vs.* I+II	39.4 *vs.* 65.3	2.404	1.401-4.123	**0.001**
PBK	Low *vs.* high	41.6 vs. 59.5	1.814	1.085-3.034	**0.023**

CI: Confidence interval; HR: hazard ratio; PBK: PDZ-binding kinase. Significant *p*-Values are shown in bold.

Table III. *Multivariate analysis of the influence of various parameters on the overall survival of patients with oral squamous cell carcinoma.*

		Overall survival			
Parameter	Category	Median, years	HR*	95% CI*	*p*-Value*
Age	≥57 *vs.* <57 Years	5.0 *vs.* 4.0	0.992	0.655-1.504	0.970
Gender	Male *vs.* female	3.7 *vs.* 8.8	1.965	0.985-3.920	0.055
Smoking	Yes *vs.* no	5.8 *vs.* 3.0	0.735	0.482-1.121	0.153
Betel quid chewing	Yes *vs.* no	8.0 *vs.* 3.7	0.544	0.312-0.950	**0.032**
Alcohol consumption	Yes *vs.* no	4.2 *vs.* 3.9	0.881	0.582-1.333	0.548
Stage	III+IV *vs.* I+II	2.6 *vs.* >8.8	2.371	1.382-4.068	**0.002**
PBK	Low *vs.* high	3.5 *vs.* >8.8	2.079	1.238-3.490	**0.006**

CI: Confidence interval; HR: hazard ratio; PBK: PDZ-binding kinase. *Adjusted for gender and stage. Significant *p*-Values are shown in bold.

respectively, Table I). A total of 47 patients were diagnosed with early-stage tumors (stages I and II), whereas 132 patients were diagnosed with advanced-stage tumors (stages III and IV). No significant correlation was observed between PBK expression and the different clinicopathological parameters, namely, age, histological type, differentiation, lymph node metastasis, and TNM stage (Table I).

Prognostic role of PBK expression in patients with OSCC with radiotherapy. The mean and median survival times were 4.9 and 4.0 years, respectively, and the overall 5-year survival rate was 46.2%. The 5-year survival rate and the median survival time were analyzed based on multiple factors: Age, gender, smoking, betel quid chewing, alcohol consumption, locally or regionally advanced stage, and PBK expression (Tables II and III). The Kaplan–Meier survival curves showed that overall survival was significantly shorter in patients with low PBK expression than in those with high PBK expression (Figure 2A). Similarly, univariate analysis showed that patients with advanced-stage OSCC had a lower 5-year survival rate than those with early-stage disease (stage III+IV *vs.* I+II: 39.4% *vs.* 65.3%, $p<0.001$, Table II). We evaluated the prognostic role of PBK overexpression through a univariate analysis, and our results showed that the 5-year survival of patients with low PBK expression was significantly shorter than that of patients with high PBK expression [hazard ratio (HR)=1.814, 95% confidence intervaI (CI)=1.085–3.034, $p=0.023$ for univariate analysis, Table II]. Table III summarizes the results of multivariate analysis with adjustment of clinicopathological factors available in this study. Staging remained a significant predictor for clinical outcome (HR=2.371, 95% CI=1.382-4.068, $p=0.002$, Table III). Moreover, PBK expression was an independent prognostic marker for radiotherapy-treated patients with OSCC (HR=2.079, 95% CI=1.238-3.490, $p=0.006$, Table III). Furthermore, as shown in Figure 2B in subgrouping of patients according to stage, PBK expression was a significant independent prognostic marker for radiotherapy-treated patients with advanced (stage III+IV) but not early stage (stage I+II) OSCC (multivariate analysis, for stage III+IV: HR=2.213, 95% CI=1.256-3.898, $p=0.006$; for stage I+II HR=1.352, 95% CI=0.381-4.802, $p=0.641$).

Discussion

As far as we are aware, this is the first study to investigate the prognostic role of PBK in patients with OSCC those with

radiotherapy. Although PBK overexpression is a poor prognostic indicator in some types of cancer (14, 15, 20, 21), our study shows that in radiotherapy-treated patients, low PBK expression was significantly associated with poor 5-year survival compared with those with high PBK expression.

In a cohort study that included 128 post-radiotherapy prostate cancer patients, Pirovano *et al*. proposed that PBK expression not only to be associated with tumor recurrence after radiotherapy but also to play a role in tumor-specific radiosensitivity (22). This intrinsic radiosensitivity leads to tumor susceptibility to ionizing radiation, resulting in better overall and disease-free survival, and recurrence rate (23). The exact role of PBK is not well established; however, several studies have suggested that PBK participates in DNA damage-repair pathways, which are regulated by insulin-like growth factor 1 and epidermal growth factor (24-27). Furthermore, Friedmann *et al*. reported that inhibition of epidermal growth factor receptor increased radiosensitivity *in vitro* (27).

Deregulation of protein kinase expression plays an essential role in cell proliferation, migration, and angiogenesis in oncogenesis. Protein kinase inhibitors (such as those of fusion breakpoint cluster region–ABL proto-oncogene 1, epidermal growth factor receptor, and B-Raf proto-oncogene, serine/threonine kinase-V600E) were successfully developed into cancer therapy. PBK, is included in the "consensus stemness ranking signature" gene list, and is a serine/threonine kinase that is up-regulated in various tumor types (5, 6, 28, 29). An increased PBK level suppresses p53 function, leading to disrupted cancer cell apoptosis and cell arrest (8). Moreover, studies have suggested that PBK participates in cytokinesis and DNA-repair sensing through phosphorylation of histone H2AX, which recruits DNA-damage response proteins to the sites of damage (9, 10). PBK prevents cancer cell death by impairing the tumor-suppressor gene *TP53*, which in turn promotes tumor cell apoptosis (11). *PBK* knockdown was shown to increase susceptibility to DNA damage and impair γ-H2AX generation in tumor cells (10). By contrast, PBK exerts protective effects on the phosphatase and tensin homolog/AKT serine/threonine kinase-dependent pathway by enhancing the antioxidant capacity in ischemic or reperfusion injury (12, 13). However, the association between PBK overexpression and clinical prognosis in OSCCs has rarely been described. Furthermore, the prognostic role of PBK expression in patients with OSCC treated with radiotherapy has not yet been reported.

Through this study, we report for the first time that poor outcome is associated with low PBK expression in patients with OSCC treated with radiotherapy. The strength of this survey might be the long-term survival period (mean=4.9 years and maximum of 9.0 years). The limitations of our study are that it is a retrospective analysis based on tissue microarrays, a small sample size, and limited size of tissue microarray from a whole tumor. More information regarding cause of patient death, and use of adjuvant or neoadjuvant chemotherapy, would better help clinicians and scientists clarify the clinical outcome. The definitive mechanism for our clinical finding is unclear. There was no information available to survey the prognostic role of PBK with *TP53* mutation status or other key mutations in cell-cycle, DNA-damage repair, and apoptosis. Although several studies have reported better outcome for patients with low PBK expression, the prognostic role of PBK overexpression in solid tumors remains debatable. Further clinical investigation regarding the association between clinical outcome and PBK expression in patients with OSCC treated with radiotherapy with large and various populations is needed for confirmation of the generalizability of our findings.

In conclusion, high PBK expression was associated with favorable prognosis in radiotherapy-treated patients with OSCC, especially for those with advanced-stage disease However, considering the limited sample size and the results that are inconsistent with the biological role of PBK, further investigation involving larger population is necessary prior to clinical application of PBK as a prognostic marker or therapeutic target.

Conflicts of Interest

The Authors declare that they have no competing interests in regard to this study.

Authors' Contributions

WNY and HFL conceived and designed this study together, WCS, WWS and CMY collected the data, WWS provided advice on the data analysis, WNY and YL wrote the article, WWS and YML revised this article All the Authors have read and approved the final manuscript.

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