Original paper

PTK7 is a molecular marker for metastasis, TNM stage, and prognosis in oral tongue squamous cell carcinoma

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The prognosis of oral tongue squamous cell carcinoma (OTSCC) is poor, and it would be beneficial to identify prognostic markers for optimization of treatment. Protein tyrosine kinase 7 (PTK7) is a receptor tyrosine kinase that is aberrantly expressed in human cancers. No exploration of the role of PTK7 in OTSCC has been reported. We detected expression of PTK7 protein in a set of tissue samples of OTSCC. The relationship between PTK7 expression and clinicopathologic parameters and overall survival of patients was analyzed. The expression level of PTK7 protein in OTSCC was increased relative to that in normal squamous cells. High expression of PTK7 was positively associated with TNM stage ($p = 0.024$), tumor differentiation ($p = 0.019$), and lymph node metastasis ($p = 0.077$). Patients with high expression of PTK7 had poor overall survival ($p = 0.058$).
Our data indicate that PTK7 protein expression is associated with the prognosis of OTSCC and may serve as a therapeutic target.

Key words: receptor tyrosine kinase 7, PTK7, CCK4, squamous cell carcinoma, tongue.

Introduction

Head and neck/oral squamous cell carcinomas (HNOSCCs) represent the sixth most common type of cancers worldwide [1]. Despite tremendous improvements in treatment by surgery, radiotherapy, and chemotherapy over the past decades, the prognosis for patients with HNOSCCs has not changed. HNOSCC is a disease that can arise in various anatomic locations, including the oral cavity, tongue, pharynx, and larynx. Oral tongue squamous cell carcinoma (OTSCC) is one of the most common types of HNOSCC and is significantly more aggressive than other forms of HNOSCC in terms of local invasion and spread. Although some of the molecular events underlying this complex disease have been identified, the pathways involved in the development and progression of squamous cell carcinoma of the tongue are still poorly understood [2, 3, 4, 5].

Protein tyrosine kinase 7 (PTK7), also known as colon carcinoma kinase-4 (CCK-4), is a member of a receptor tyrosine kinase (RTK) family that is conserved among human, *Drosophila*, Japanese puffer fish, *Hydra*, and chicken. PTK7 is an evolutionarily conserved transmembrane protein containing seven immunoglobulin domains, a transmembrane domain, and a catalytically inactive kinase domain [6, 7, 8]. PTK7 is a Wnt co-receptor and an important regulator of planar cell polarity (PCP) and directional cell motility in embryogenesis and vertebrate development [9, 10, 11]. The PCP signaling pathways control cell mobility and cellular polarity, re-

sulting in modification of the cytoskeleton. Deregulation of PCP can cause various pathologic disorders, including cancer. Well-known regulators of PCP include Wnt ligands and Frizzled receptors, which activate the Dishevelled adaptor at the plasma membrane and initiate the so-called Wnt pathway, either in its canonical (β-catenin-dependent) or non-canonical (β-catenin-independent) form [12, 13].

Overexpression of PTK7 has been documented in several human cancers, including colon, gastric, and esophageal cancer and acute myelogenous leukemia. Overexpression of PTK7 is associated with an adverse clinical outcome in colorectal cancer, and high expression of PTK7 is significantly associated with tumor invasiveness and poor prognosis in intrahepatic cholangiocarcinoma. Interestingly, we previously found that the expression of PTK7 protein was decreased in epithelial ovarian carcinomas with poor prognosis [14].

In this study, we examined the expression of PTK7 protein in a set of formalin-fixed paraffin-embedded tissue samples of OTSCC using a PTK7-specific polyclonal antibody and analyzed the relationship between the expression of PTK7 and clinicopathologic parameters and overall survival of patients.

Material and methods

Patients and clinicopathologic variables

The study cohort consisted of 64 consecutive patients with OTSCC who underwent surgery from 2001 to 2011 in Nanjing Stomatological Hospital, China. Patient age ranged from 20 to 85 years (mean age 57 years, SD = 11.7), and 44 patients had high-grade disease and 20 had low-grade disease. None of the patients received preoperative chemotherapy or radiation therapy. All hematoxylin and eosin-stained slides were reviewed by two experienced pathologists to verify the diagnosis, histologic grade, and stage. HPV infection was detected by the PCR method. The patients were followed up until April 2012. The records of patients who were alive at follow-up or who did not die of the disease were considered to be censored. This investigation was performed after obtaining approval from the Ethics Committee of Nanjing Stomatological Hospital, China. Informed consent was obtained from each patient.

Immunohistochemistry

Sections from surgical specimens were fixed in 10% formalin and embedded in paraffin. Immunohistochemical staining was carried out according to a standard method. Briefly, each 4-μm tissue section was deparaffinized and rehydrated through a graded ethanol series. The sections were autoclaved in 10 mM citrate buffer (pH 6.0) at 120°C for 2 min for antigen retrieval and then cooled to 30°C and washed with phosphate-buffered saline (PBS, pH 7.3). Endogenous peroxidase were quenched with aqueous 3% H_2O_2 for 10 min and the sections were washed with PBS and incubated at 4°C overnight with primary rabbit polyclonal anti-PTK7 antibody (1 : 500 dilution; Abgent, San Diego, CA, USA). The sections were incubated with secondary antibody (Dako REAL EnVision Detection System, Dako, UK) for 20 min at room temperature. After counterstaining, the slides were washed with PBS, dehydrated, cleared in xylene and mounted in neutral balsam. Primary antibody was replaced with antibody diluent for negative controls. Samples of normal fallopian tubes with known positivity were used as positive external controls.

PTK7 staining was independently evaluated for immunoreactivity according to the scoring criteria below by two pathologists who were double-blinded to clinical data. Immunoreactivity score was determined according to the intensity of cytoplasmic staining where 0 = no staining, 1+ = weak, 2+ = moderate, and 3+ = strong. The expression of PTK7 was defined as negative (0 and 1+) or positive (2+ and 3+).

Follow-up

Follow-up data were collected from the patients' records and telephone interviews of patients. Overall survival (OS) was defined as the time from surgery to the date of death. The median follow-up time was 37 months (range: 7-115 months).

Statistical analysis

The χ^2 test was used to assess the relationship between PTK7 protein expression and clinicopathologic parameters. Survival curves were constructed using the Kaplan-Meier method, and differences between the curves were compared using the log-rank test. P-values < 0.05 (two-sided) were considered statistically significant. All analyses were performed using SPSS software (version 16.0, Chicago, IL, USA).

Results

PTK7 expression in OTSCC and its correlation with clinicopathologic features

The expression of PTK7 protein in OTSCC tissue specimens was determined by immunohistochemical staining. As shown in Figs. 1 and 2, PTK7 staining was localized predominantly in the cytoplasm and to a lesser extent in the nucleus. PTK7 protein was differentially expressed among the 64 samples of OTSCC; 35 (54.7%) samples showed negative or weak (0/+) staining with anti-PTK7 antibody, whereas 29 samples (45.3%) showed moderate (2+)

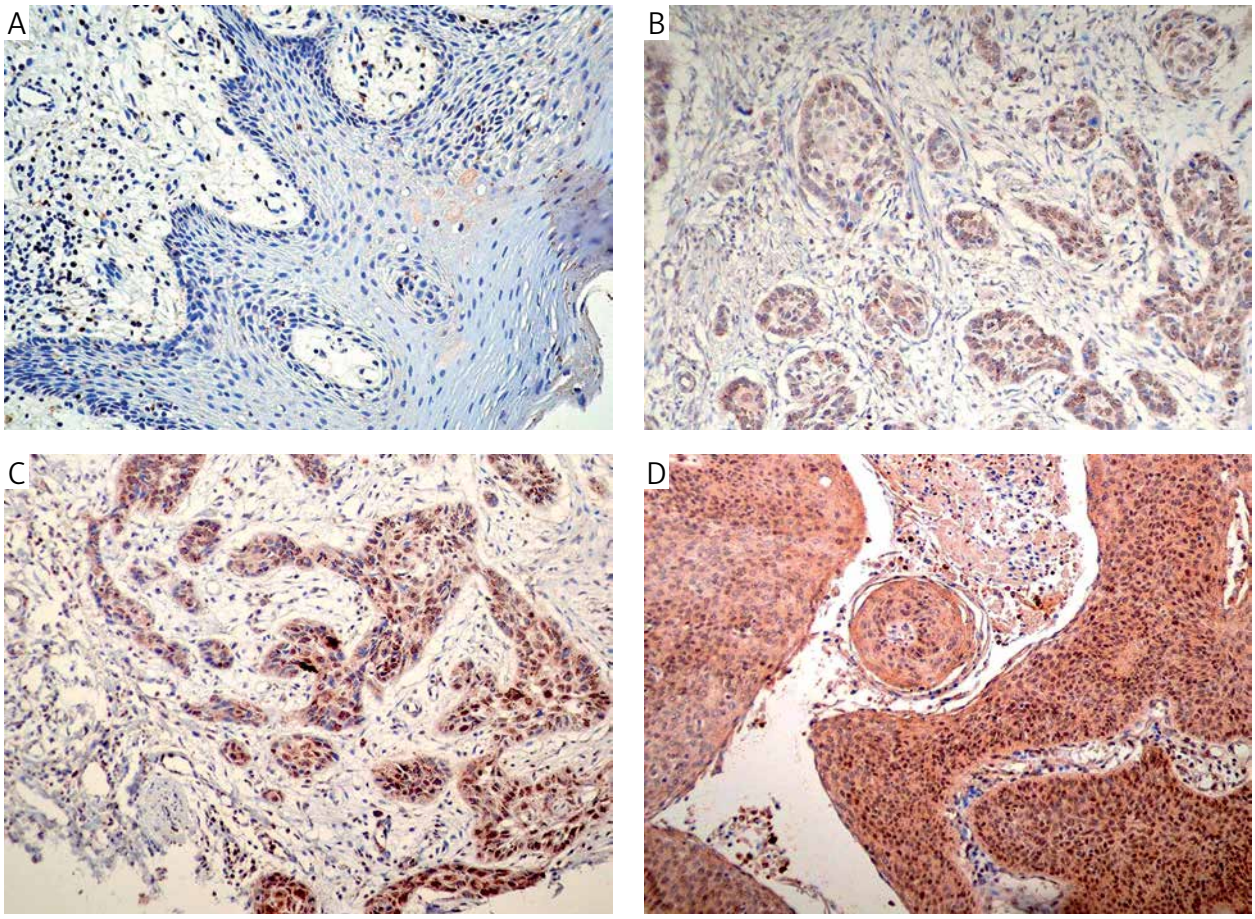

Fig. 1. Increased expression of PTK7 protein in OTSCC. A) Negative expression of PTK7 in normal squamous cells. B) Weak expression of PTK7 in OTSCC. C) Moderate expression of PTK7 in OTSCC. D) High expression of PTK7 in OTSCC (magnification 200×)

or strong (3+) staining. Thus, positive expression of PTK7 was observed in 45.3% (29/64) of cases of squamous cell carcinoma of the tongue. Analysis of the relationship between PTK7 expression and pathologic parameters revealed that expression of PTK7 was positively associated with lymph node metastasis ($p = 0.077$), TNM stage ($p = 0.024$) and differentiation ($p = 0.019$) (Table I).

Association between PTK7 expression and overall survival in patients

Using the follow-up data of the 64 patients in conjunction with the results from PTK7 IHC staining experiments, we showed that patients with high PTK7 expression (2+ and 3+) had a poor overall survival (OS) compared with patients with negative (0) or weak PTK7 expression (1+) ($p = 0.058$, Fig. 3).

Discussion

PTK7 expression has been evaluated in various human cancers and, interestingly, was found to be increased in some cancer types and decreased in other types. Lhoumeau *et al*. examined PTK7 expression by immunohistochemistry in tumoral tissue and matched normal mucosae using a clinically annotated tissue microarray (TMA) from 192 consecutive cases of colorectal cancer (CRC) [15]. The relationship between expression of PTK7 and clinicopathologic features and outcome of patients was analyzed. PTK7 was significantly upregulated in 34% of CRC tissues compared with matched healthy mucosa. PTK7 overexpression was significantly associated with reduced metastasis-free survival in patients with non-metastatic disease. They further showed that PTK7 depletion by specific shRNA in two CRC cell lines (HCT116 and HCT15) reduced cell migration but did not affect cell proliferation or resistance to drugs. Downregulation of PTK7 in a xenograft mouse model led to reduced tumor growth, whereas introduction of PTK7 into PTK7-negative cancer cells led to increased metastatic events *in vivo*. The authors concluded that PTK7 is a potential prognostic biomarker and a novel therapeutic target in CRC.

The expression and role of PTK7 in intrahepatic cholangiocarcinoma (ICC) were investigated by Jin *et al*. [16]. They found that cells with high PTK7 expression exhibited higher proliferation, DNA

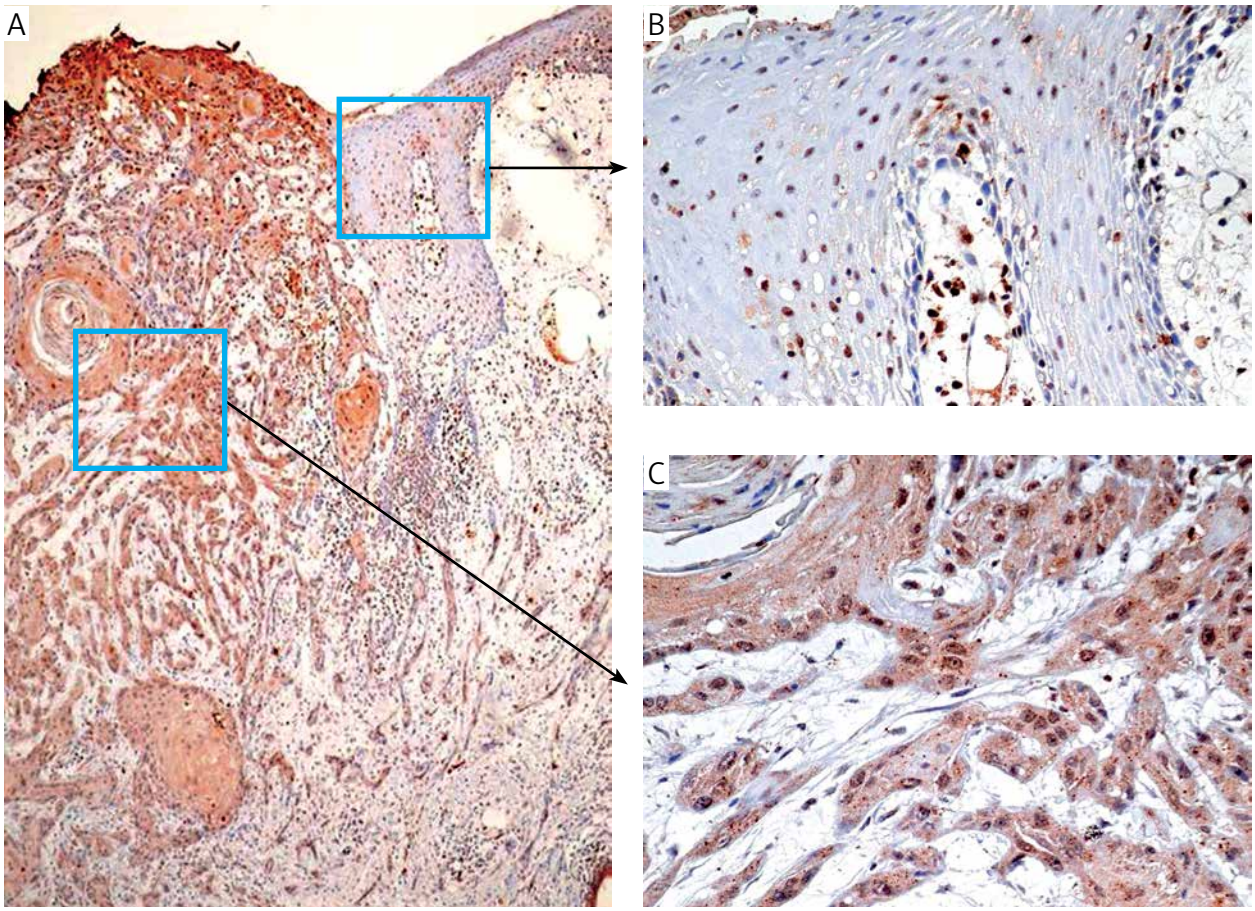

Fig. 2. A) Representative example of decreased expression of PTK7 in OTSCC. B) High-power view of normal squamous cells in panel A. C) High-power view of squamous cell carcinoma cells in panel A (magnification 200×)

Table I. The relationship between the expression of PTK7 protein and clinicopathological parameters in tongue squamous cell carcinoma

Parameters		PTK7 protein expression		P value
		0/1+	2+/3+	
Sex	male	16	14	1.000
	female	19	15	
Age (years)	< 60	22	16	0.613
	≥ 60	13	13	
TNM stage	I+II	20	8	0.024
	III+IV	15	21	
Differentiation	high	18	6	0.019
	moderate/poor	17	23	
Lymph node	no	23	12	0.077
	yes	12	17	
Smoking	no	20	16	0.135
	yes	15	13	
HPV infection	no	21	12	0.209
	yes	14	17	

synthesis, invasion, and migration abilities. Knockdown of *PTK7* with siRNA in high-expression cells resulted in impairment of invasion, migration, and DNA synthesis through the regulation of several cell cycle-related proteins. PTK7 expression was higher in human ICC tissue than in the normal bile duct. Moreover, patients with low expression of PTK7 had longer disease-free survival and overall survival than those with high expression.

Shin *et al.* studied the expression of PTK7 in esophageal squamous cell carcinoma (ESCC) [17]. PTK7 was significantly upregulated in tumor tissue samples and its expression was inversely correlated with overall survival. *In vivo* knockdown of *PTK7* inhibited proliferation, survival, wound healing, and invasion of ESCC cells.

All of the above studies indicate that PTK7 has an oncogenic role in human cancers and might be used as a prognostic marker for cancer patients.

However, in other types of human cancer PTK7 was revealed to function as a tumor suppressor. Easty *et al*. studied the expression of PTK7 in melanoma cell lines and biopsies [18]. *PTK7* mRNA was detected in only 54% of melanoma cell lines and 20% of melanoma biopsies, and loss of expression of PTK7 was observed in metastatic melanomas. Lin *et al.* assessed the expression of PTK7 in 201 gastric cancer samples using immunohistochemistry [19]. PTK7 expression was detected in 56.7% of gastric cancer patients, and was significantly associated with well-differentiated tumor status and favorable overall survival and disease-free survival. We previously reported that PTK7 expression is decreased in epithelial ovarian carcinoma with poor prognosis [14]. Expression of PTK7 was detected in 92% of normal fallopian tube epithelium and 45% of epithelial ovarian tumor tissues. Expression of PTK7 was significantly associated with clinical stage and metastasis in ovarian borderline serous tumors. Patients with negative expression of PTK7 had a poor outcome. These results indicate that PTK7 may be a tumor suppressor in ovarian serous carcinoma.

In the present study, we found that high expression of PTK7 was more often detected in oral tongue squamous cell carcinoma patients with lymph node metastasis, advanced TNM stage, and a poorly differentiated tumor. Patients with high expression of PTK7 had poor overall survival. To our knowledge, this is the first report of the expression of PTK7 in oral tongue squamous cell carcinoma. Carcinoma of the tongue is a challenging disease; it has a poor prognosis at an advanced stage, and even tumors that are discovered at an early stage can have a fatal course. According to the National Comprehensive Cancer Network (NCCN) Guidelines for Cancer of the Oral Cavity (2016, version 2), stage I carcinoma of the tongue is treated with wedge excision only, whereas

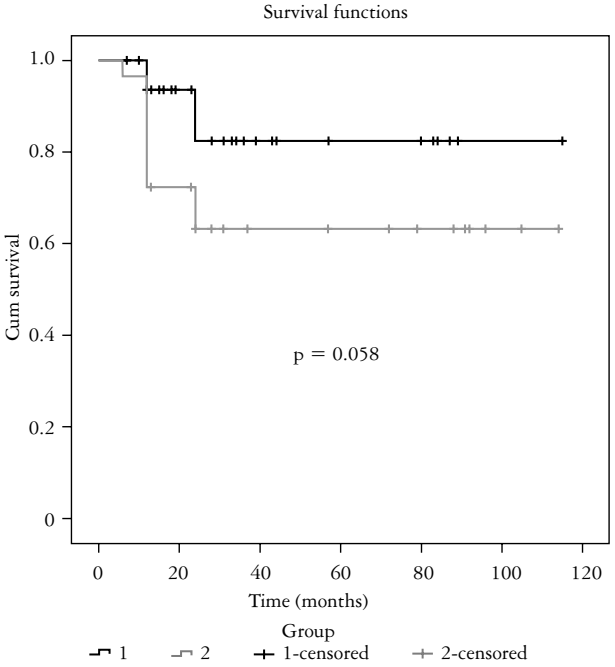

Fig. 3. Kaplan-Meier plots of overall survival show that patients with high PTK7 expression had shorter survival than those with negative PTK7 expression (group 1: score 0 or 1+ for PTK7 immunoreactivity; group 2: score 2+ and 3+ for PTK7 immunoreactivity)

more advanced tumors (stages II-IV) are treated with preoperative irradiation followed by more extensive surgery. Nguyen *et al*. reported their experience in providing effective and well-tolerated 0-7-21 palliative radiotherapy to patients with head and neck cancers [20]. However, among stage I tongue carcinomas there is a subgroup of tumors that are prone to lymph node metastasis and local recurrence. If this more aggressive tumor behavior could be predicted, patients who are at increased risk of lymph node metastasis and recurrence could be subjected to extended treatment, whereas those with less aggressive carcinomas could be spared the morbidity associated with such treatment. Many attempts have been made to predict the outcome for tongue carcinomas through histopathologic studies and molecular biology analysis [21, 22, 23, 24].

Receptor tyrosine kinases are key mediators of signaling pathways in cells, which have been shown to be involved in regulation of normal cellular processes and also play an important role in the development, progression and spread of cancers. Recently, some of the receptor tyrosine kinases including HER2, EGFR, and VEGFR have become key targets for cancer therapy. PTK7 is a member of the receptor tyrosine kinases. Our findings suggest that PTK7 plays an oncogenic role in oral tongue squamous cell carcinoma. The inhibition of PTK7 expression could be a new therapeutic target for oral tongue squamous cell carcinoma.

Conclusions

In summary, our data indicate that high expression of PTK7 was significantly associated with lymph node metastasis, advanced TNM stage, poor differentiation status, and poor overall survival of patients with oral OTSCC. PTK7 protein expression was associated with prognosis of squamous cell carcinoma of the tongue and may serve as a prognostic marker and a potential therapeutic target.

This investigation was performed with approval from the Ethics Committee of Nanjing Stomatological Hospital, China (reference number: 2015 KY-087).

Informed consent was obtained from each patient. The documents are available if required.

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The authors declare no conflict of interests.

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