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RACK1 is an organ-specific prognostic predictor in OSCC

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ABSTRACT

Objectives: This study aims to verify that RACK1 is an organ-specific prognostic predictor in patients with oral squamous cell carcinoma (OSCC).

Experimental design: The RACK1 expression level was assessed by immunohistochemistry (IHC) in a total of 342 OSCC patients from 3 independent cohorts. The multivariate hazard ratios for Overall Survival (OS) was determined by Cox proportional hazards regression model. OS was analyzed in 460 Head Neck Squamous Cell Carcinoma (HNSCC) patients from TCGA data set. The expression level of RACK1 was analyzed in 60 cases multiple organ tissue microarrays representing both normal and cancer tissues by IHC, and in TCGA database of mRNA abundance in cancers and paired normal tissues.

Results: The median follow-up times of patients in the study was 74, 52, and 78 months. High expression of RACK1 was identified in tumors from 103 of 151 patients (68.2%), 51 of 83 patients (61.4%), and 59 of 108 patients (54.6%). Compared with low expression, high expression of RACK1 was strongly associated with worse OS, with HR of 0.5995 (95% CI, 0.3929 to 0.9147; P = 0.0176), 0.4402 (95% CI, 0.2321 to 0.8348; P = 0.0120), and 0.5010 (95% CI, 0.2886 to 0.8699; P = 0.0141). This finding is consistent with TCGA HNSCC data (P = 0.0276). Tissue microarrays analyses showed different protein expression level of RACK1 in multiple human carcinomas and this finding is consistent with the TCGA database analysis of RACK1 mRNA abundance.

Conclusion: Our findings demonstrated that RACK1 is a good independent organ-specific predictor of the risk of death in OSCC.

Introduction

OSCC is one of the most common subtypes of HNSCC, with an estimated 300,000 new cases and 140,000 deaths occurring worldwide each year [1]. The five-year survival rate of patients with OSCC is only about 50%, despite recent advances in diagnosis and treatment [2]. Now, the most significant factors to affect outcome of patients with OSCC are still the classical clinic pathological parameters of tumor such as tumor stage, and clinical TNM stage, which are not possible to be good predictors of the risk of death. It is critical to identify an effective prognostic predictor for OSCC.

RACK1, a 36 kDa intracellular receptor for the protein kinase C family, is a member of the Trp-Asp^{40} (WD40)-repeat protein family, and has homology with the G protein β subunit of transducin [3]. RACK1 plays a pivotal role in a wide range of biological responses, including cell growth, migration, differentiation, signal transduction, and immune response [4]. In our previous study, we found that RACK1 was a predictor for poor clinical outcome in OSCC [5]. However, inconsistent results are also shown in in other studies. For example, in human colon cells, RACK1 was found could induce apoptosis by blocking Src activation of the Akt cell survival pathway [6]. Besides, RACK1 could promote apoptosis by promoting Bax oligomerization and dissociating the complex of Bax and Bcl-XL [7]. Because of those diametrically opposed conclusions, and only one cohort used in our previous analysis of the relationship between RACK1 and the development and progression of OSCC, we explored the protein in three independent cohorts from three centers. Besides, an independent cohort of 460 patient specimens obtained between 1992 and 2013 in the TCGA database (http://tcga-data.nci.nih.gov/tcga/) was also used as an external validation cohort to validate the prognostic value of RACK1 in patients with HNSCC. Unlike previous analysis of one center, our analysis of three centers and TCGA database was more credible for excluding regional heterogeneity. We confirmed that RACK1 was negatively correlated with the prognosis of patients with OSCC. Besides, the expression level of RACK1 was analyzed in tissue microarrays by IHC.

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Methods

Patient samples

The Institutional Review Boards of the West China Hospital of Stomatology, Sichuan University, the General Hospital of the People's Liberation Army (PLAGH) and Guangdong Provincial Stomatological Hospital approved this study. The study was approved by the ethics committee of the West China Hospital of Stomatology, PLAGH and the Guangdong Provincial Stomatological Hospital and was conducted in agreement with the Helsinki Declaration. Written informed consent was provided by all participants at baseline and during follow-up.

A total of 342 postoperative patients with primary OSCC tumors received regular follow-up. Follow-up visits entailed at least a medical history and clinical examination. In addition to scheduled visits, all patients could initiate visits if they were concerned that they had recurrence or a new primary tumor. The survival time of each patient was calculated from the day of surgery until the time of cancer-related death or the end of the follow-up period, death for other reasons led to censoring of data. The detailed information of three cohorts was described in Supplemental Patients and Methods.

An independent cohort of 460 patient specimens obtained between 1992 and 2013 in the TCGA database (http://tcga-data.nci.nih.gov/tcga/) was used as an external validation cohort to validate the prognostic value of RACK1 in patients with HNSCC (Table 1).

Tissue microarray

The cohort of 60 pairs of human multi-organ cancer tissues and their corresponding adjacent noncancerous tissues microarrays was purchased from Shanghai Outdo Biotech. Co. Ltd (Shanghai, China) and approved by the Ethics Committee of Taizhou Hospital.

Immunohistochemical assay and analysis

Sections were deparaffinised and rehydrated. The slides were then heated in a 100°C water bath for 3 min in a 0.01 M citrate buffer solution at pH 6.0, and cooled to room temperature. After quenching the endogenous peroxidase activity with 3% H_2O_2 (in absolute methanol) for 20 min, the sections were treated for 30 min at 37°C with 5% bovine serum albumin (Sigma, St. Louis, MO, USA) to block non-specific staining. Sections were incubated overnight in 4°C with the anti-RACK1 antibody (as previously mentioned, 1:50 dilution). Binding was detected with ChemMate DAKO EnVision Detection Kit (DAKO, Copenhagen, Denmark). Finally, the sections were counterstained with Mayer's hematoxylin. The staining was assessed by three independent investigators without any knowledge of the clinic-pathological data. The following criteria were used to score the staining, first was staining intensity: 0-no detectable staining, 1-light yellow, 2-deep yellow, or 3-brown; and the second criterion was staining proportion: 0 (<5%), 1 (5–25%), 2 (25–50%), 3 (51–75%) or 4 (>75%). The product of the two scores was considered as the final score, and it was divided into two levels: 0–6 score (RACK1 low expression) and more than 6 (RACK1 high expression). The score of staining intensity was acceptable if two or more investigators independently defined it as such.

Statistical analysis

Baseline characteristics among the patients were compared using the mixed linear model for continuous variables. OS was estimated using the Kaplan–Meier method, with a log-rank test in a univariate analysis. Model discrimination was measured using C Statistic for survival analysis. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC). Unless stated otherwise, two-sided $P < 0.05$ were considered significant.

Results

Patient and disease characteristics

A total of 342 patients from three independent cohorts (151, 83, and 108 patients in CD cohort, BJ cohort, and GZ cohort, respectively) were included in this study. All patients were treated with curative intent. Some of these patients had been treated by radiotherapy and/or chemotherapy. The mean age and gender distribution were comparable across the three cohorts. The median durations of follow up in the cohorts were 74, 52, and 78 months, respectively. High expression of RACK1 was observed in 68.2%, 61.4%, and 54.6% of the patients in the CD cohort, BJ cohort and GZ cohort, respectively (Table 1).

Table 1
Baseline characteristics of the patients with OSCC.

Characteristic	CD Cohort (N = 151) number (%)	P value	BJ Cohort (N = 83) number (%)	P value	GZ Cohort (N = 108) number (%)	P value
Gender						
Male	107 (70.9)	0.714	65 (78.3)	0.847	41 (38.0)	0.875
Female	44 (29.1)		18 (21.7)		67 (62.0)	
Age-yr (mean ± sd)	61.07 ± 12.58		60.89 ± 12.73		61.46 ± 12.45	
<60	68 (45.0)	0.515	40 (48.2)	0.577	44 (40.7)	0.596
≥60	83 (55.0)		43 (51.8)		64 (59.3)	
Smoking status						
Smoker	74 (49.0)	0.272	41 (49.4)	0.247	68 (63.0)	0.33
Nonsmoker	77 (51.0)		42 (50.6)		40 (37.0)	
Tumor stage						
T1-2	94 (62.3)	0.016	52 (62.7)	0.219	66 (61.1)	0.008
T3-4	57 (27.7)		31 (37.3)		42 (38.9)	
Clinical TNM stage						
I-II	77 (51.0)	0.005	37 (44.6)	0.3	46 (42.6)	0.084
III-IV	74 (49.0)		46 (55.4)		62 (57.4)	
Radiotherapy or Chemotherapy						
Yes	85 (56.3)	0.804	27 (32.5)	0.799	70 (64.8)	0.866
No	66 (43.7)		56 (67.5)		38 (35.2)	
RACK1						
Low expression	48 (31.8)	0.018	32 (38.6)	0.012	49 (45.4)	0.014
High expression	103 (68.2)		51 (61.4)		59 (54.6)	