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Original Article

Associations between proteasomal activator PA28γ and outcome of oral squamous cell carcinoma: Evidence from cohort studies and functional analyses

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SUMMARY

Background: PA28γ was suggested to play a role in malignant progression. This paper aimed to investigate the association between PA28γ and the prognosis of oral squamous cell carcinoma (OSCC) in cohort studies.
Methods: The PA28γ expression level was assessed by immunohistochemistry in a total of 368 OSCC patients from three independent cohorts. The Cox proportional hazards regression model was used to determine multivariate hazard ratios for Overall Survival (OS). Model discrimination was measured using C Statistic. Additionally, OS was analyzed in Head Neck Squamous Cell Carcinoma (HNSCC) patients from The Cancer Genome Atlas (TCGA) data set. Functional analyses were conducted both in-vitro and in-vivo.
Findings: The median follow-up times of patients in the three studies were 60, 52, and 51 months. High expression of PA28γ was identified in tumors from 179 of 368 patients (48.6%). Compared with low expression, high expression of PA28γ was strongly associated with worse OS, with relative risks of 5.14 (95% CI, 2.51–10.5; $P < 0.001$), 2.82 (95% CI, 1.73–4.61; $P < 0.001$), and 3.85 (95% CI, 1.59–9.37; $P = 0.003$). PA28γ expression was also associated with disease-free survival in all three cohorts ($P < 0.005$). These findings are consistent with TCGA HNSCC data ($P < 0.006$). The prediction of all-cause mortality was significantly improved when PA28γ was added to the traditional clinical factors (Model 3, C statistic value: 0.78 VS 0.73, $P = 0.016$). In functional analyses, we found that PA28γ silencing dramatically inhibited the growth, proliferation and mobility of OSCC cells in vitro and reduced tumor growth and angiogenesis in tumor-bearing mice.
Interpretation: PA28γ overexpression is associated with adverse prognosis in patients with OSCC. The aberrant expression of PA28γ may contribute to the pathogenesis and progression of OSCC.
Research in context: OSCC is one of the most common HNSCC, which have a high lethally rate. However, few prognostic markers have been applied in the clinical practice. We found that PA28γ in OSCC tumor tissues were significantly high expression than those in normal tissues. As the results of the three cohorts from two independent research centers and from an additional validation cohort from a US population in the TCGA dataset, we demonstrate PA28γ is a good predictor of the risk of death in OSCC. Meanwhile, we demonstrate PA28γ have a potential role in OSCC tumorigenesis.

1. Introduction

OSCC is one of the most common HNSCC, with an estimated 260,000 new cases and 120,000 deaths worldwide each year (Jemal et al., 2011). Despite recent advances in diagnosis and treatment, the 5-year survival rate of patients with OSCC is no more than 50% (Panzarella et al., 2014). Over the past two decades, numerous prognostic and predictive markers for clinical outcomes in OSCC have been proposed (Ratajczak-

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Wrona et al., 2013); however, few have been applied in clinical practice due to the non-reproducibility of the initial findings (Choi and Myers, 2008; Principe et al., 2013). To date, the classical clinic pathological parameters of tumor such as primary site, tumor stage, lymph nodal stage and clinical TNM stage remain the most significant factors to affect outcome of patients with OSCC. However, it is impossible to predict patients at a high risk of death mainly based on these parameters. Therefore, it is critical to identify novel and effective prognostic predictors and therapeutic targets for treating this common malignancy.

In eukaryotic cells, proteasomes play an essential role in intracellular proteolysis and are involved in the control of most biological processes through regulated degradation of key proteins. PA28 is a member of a unique family of proteasomal activators that has the ability to stimulate the proteolytic activity of the 20S core proteasome independent of ubiquitination and ATP (Li et al., 2007). Unlike PA28α and PA28β, PA28γ (also known as Ki antigen, 11Sγ, or REGγ) localizes in the nucleus and forms a homo-heptamer (Kloetzel and Ossendorp, 2004; Rechsteiner et al., 2000; Rivett and Hearn, 2004). PA28γ, regulated by MEKK3, B-RAF, caspase-3/7 and targeted by miR-7-5p, is a multifunctional protein that is involved in the degradation of important regulatory proteins, such as SRC-3, PTTG1 and cyclin-dependent kinase inhibitors p21/16/19 in an ubiquitin- and ATP-independent manner, and has been implicated in the regulation of cell cycle progression (Li et al., 2007; Araya et al., 2002; Chen et al., 2007; Ying et al., 2006; Shi et al., 2015). Moreover, PA28γ-deficient mice have been shown to exhibit growth retardation (Barton et al., 2004). Several targets of PA28γ have been identified in recent years, suggesting that it plays important roles in angiogenesis, hepatic lipid metabolism, infectious diseases and premature aging (Liu et al., 2014; Dong et al., 2013; Yan et al., 2014; Li et al., 2013). PA28γ is over-expressed in some cancer tissues, suggesting that this protein may also have a potential role in tumorigenesis (Wang et al., 2011; Roessler et al., 2006). Some studies found that PA28γ may facilitate the turnover of the tumor suppressor p53 by promoting murine double minute 2 (MDM2)-mediated p53 ubiquitination (He et al., 2012; Zhang and Zhang, 2008) and PA28γ could take part in the ATM-DBC1-SIRT1 axis induced p53-dependent apoptosis (Magni et al., 2014). Recently researchers found that mutant p53 (p53-R248Q) could up-regulate PA28γ in endometrial cancer (Wang et al., 2015), thus, there is an auto-regulatory feedback loop between p53 and PA28γ (Wan et al., 2014). Nevertheless, the mechanism by which PA28γ exerts its effects on tumor cells remains unclear.

In our previous study, we conducted a comprehensive proteomic analysis to identify candidate biomarkers in OSCC (Wang et al., 2008). Expression levels of 52 proteins in OSCC tumor tissues were significantly different from those in normal tissues (Wang et al., 2009). One of these proteins was PA28γ. The bioprocesses and interaction network analysis indicated that PA28γ might play an important role in malignant transformation. Given these findings, we hypothesized that PA28γ may be involved in malignant development and progression of OSCC and would have effect on the prognosis of this disease. To test this hypothesis, we first explored the protein expression profile of PA28γ and its relations with the outcome of OSCC patients in three independent cohorts from two centers. Then, we constructed models to predict death of patients with OSCC using PA28γ individually and jointly with other prognostic factors identified in these three cohorts. Finally, we investigated the effects of PA28γ on the biological behavior of OSCC cells both in vitro and in vivo.

2. Methods and patients

2.1. Patients

The Institutional Review Boards of the West China Hospital of Stomatology, Sichuan University and Guangdong Provincial Stomatological Hospital approved this study. The study was approved by the ethics committee both of the West China Hospital of Stomatology 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 368 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

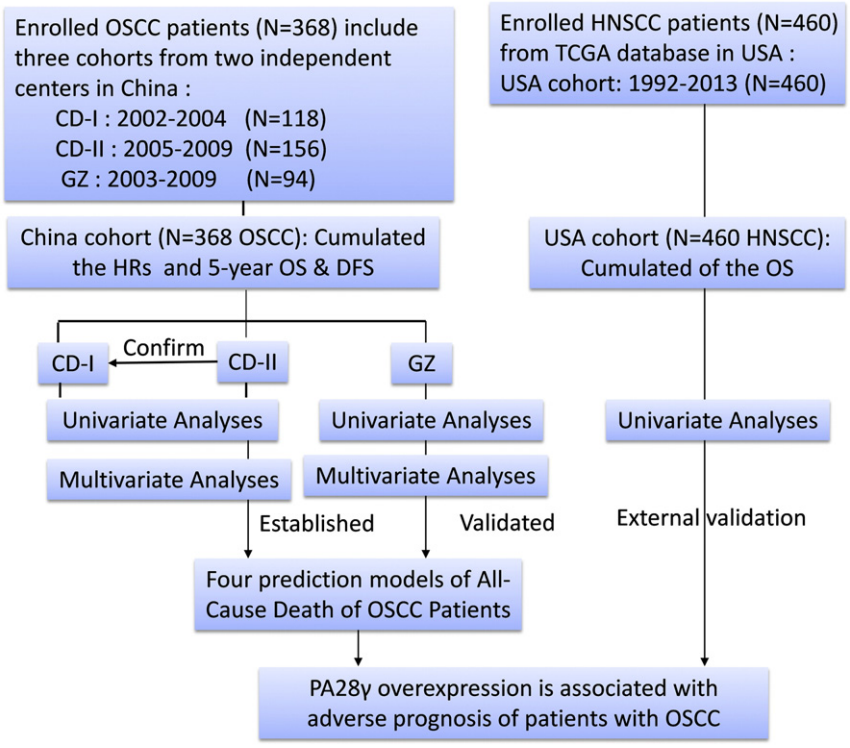

Fig. 1. Study flow chart.