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High expression of TROP2 is correlated with poor prognosis of oral squamous cell carcinoma

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ABSTRACT

Human trophoblastic cell-surface antigen 2 (TROP2) is a cell surface glycoprotein that exhibits high expression in various carcinomas but low or no expression in normal tissues. High TROP2 expression plays an important role in promoting tumor development and aggressiveness, which is correlated with reduced patient survival. However, there are few studies regarding TROP2 in relation to both oral squamous cell carcinoma (OSCC) and oral potentially malignant lesions. The expression of TROP2 protein and mRNA was investigated in OSCC tissues, oral potentially malignant lesion tissues, and normal oral tissues using immunohistochemistry and quantitative real-time polymerase chain reaction (qRT-PCR). The association between TROP2 expression and clinicopathological characteristics of OSCC was also analyzed, and the prognostic value of TROP2 was evaluated. The expression of TROP2 protein and mRNA were both higher in OSCC tissues than in oral potentially malignant lesion tissues or normal oral tissues. Positive TROP2 expression was related to differentiation, lymph node metastases, TNM stage, perineural infiltration, and vascular invasion. Poor overall survival was associated with high TROP2 expression and other factors associated with poor overall survival including poor differentiation, lymph node metastasis, TNM stage, vascular invasion, and perineural invasion in univariate analyses. TROP2 expression as well as TNM stage and vascular invasion were independent prognostic factors associated with the overall survival of OSCC patients in multivariate analyses. In summary, High TROP2 expression is associated with poor overall survival and serves as an independent prognostic factor in OSCC. The results suggest that TROP2 expression could be an effective prognostic biomarker for OSCC.

1. Introduction

Oral cancer is one of the prevalent malignant cancers worldwide. Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, with 90% of oral cancers originating from squamous cells [1,2]. It is widely known that most oral carcinomas evolve from oral potentially malignant conditions or lesions such as oral lichen planus, oral leukoplakia, or oral erythroplakia. The progression of oral carcinogenesis is multi-stepped and includes the process of genes being amplified or dysregulated [3]. Most OSCC are close to important tissues

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and organs, which severely restricts surgery for safety reasons. Neck lymph node metastasis and invasion often occur because of the frequent mechanical movement of the tongue and the position of abundant blood vessels and nerves in the maxillofacial tissues (all rich lymphatic vessels), so the prognosis is often poor.

Despite large amounts of available data reporting on the diagnosis and combined treatment of OSCC with surgery, radiotherapy, and chemotherapy [4], the 5-year survival rate remains below 60% [5]. In recent years, few molecular targets have been identified in OSCC, which makes it difficult to develop targeted therapy for OSCC. Thus, the identification of molecular targets is particularly important in the effective treatment for OSCC.

Trophoblast antigen 2 (TROP2), also termed tumour-associated calcium signal transducer 2 (TACSTD2), membrane component 1 surface marker 1 (M1S1), epithelial glycoprotein 1 (EGP1) and gastrointestinal antigen 733-1 (GA733-1), is a type I transmembrane surface glycoprotein of 35 kDa with an extracellular domain, a single transmembrane domain, and a short intracellular tail [6,7]. The TROP2 gene has been mapped to chromosome 1p32, an intronless product of 323 amino acids with a total length of 2080bp. TROP2 is an important factor in trophoblasts in the placenta and in many organs during embryonic and fetal development. TROP2 expression promotes fetal lung growth and development. TROP2 overexpression increases cell proliferation of fetal rat lung fibroblasts, whereas knock-down of TROP2 with siRNA decreases proliferation in vitro study [8]. TROP2 is also studied during the development of the intestines and kidney. High TROP2 expression maintains the intestinal progenitor cells in an undifferentiated state and proliferates epithelial progenitor cells at E14 in the duodenum. During the development of the kidney, TROP2 expression is high levels in the ureteric bud epithelial stem cells and low levels in the ureteric bud cells [9,10].

High TROP2 expression has also been observed in most human cancers including ovarian [11], gastric [12,13], colorectal [14], pancreatic [15], and laryngeal [16] cancer types, while its protein expression is low or absent in normal tissues [17,18]. Expression of this protein at high levels confers oncogenic properties and it is able to induce the adhesion, invasion and migration of tumor cells. Studies have shown that TROP2 is highly expressed in prostate cancer cells, resulting in decreased cell adhesion to fibronectin, via increased association of receptor for activated C kinase 1(RACK1) to integrin β1. TROP2 promotes the translocation of RACK1 from the cytoplasm to the cell membrane, which is closely connected with integrin β1 [19]. Furthermore, TROP2 promotes the proliferation, migration, and metastasis of gallbladder cancer cells by regulating the phosphatidylinositol 3 kinase/ protein kinase B pathway and inducing epithelial-mesenchymal transition, resulting in tumor progression and metastasis [20]. These findings indicate an important role for TROP2 in tumor invasiveness, progression, and recurrence, which are related to poor prognosis [21]. TROP2 is also known to activate some signaling pathways, which subsequently result in the expression of downstream effectors, such as Ca^{2+}, gene of phosphate and tension homology deleted on chromosome ten(PTEN), cyclinD1 and B-cell lymphoma-2(Bcl-2), depending on the phosphorylation site in the membrane region of TROP2, causing tumor aggressiveness [22,23].

Although TROP2 expression has been investigated in many other cancers and is known to play a role in regulating carcinoma growth, its biological function and prognostic role in OSCC remains unclear. This study aimed to investigate the expression of TROP2 in OSCC tissues, oral potentially malignant lesion tissues, and normal oral tissues, and the correlation between TROP2 expression and various clinicopathological features, as well as patient prognostic outcomes including overall survival and disease-free survival. TROP2 mRNA expression was also detected in fresh frozen oral tissues via quantitative reverse transcription–polymerase chain reaction (qRT-PCR).

2. Materials and methods

2.1. Patient samples

Paraffin-embedded oral tissue samples were obtained from 443 cases (187 OSCC tissues, 76 hyperplasia tissues, 102 oral potentially malignant lesion tissues, and 78 normal oral tissues) between 2008 and 2012. And 102 oral potentially malignant lesion tissues included 44 mild dysplasia, 30 moderate dysplasia, 28 severe dysplasia. All oral tissue samples were obtained from the Department of Pathology, Affiliated Hospital of Stomatology, Nanjing Medical University. All patients in our study had not received radiation therapy, chemotherapy, or immunotherapy before surgery or biopsy. The clinicopathological information in each case, including age, sex, tumor site, tumor size, lymph node metastases, metastasis distance, differentiation, vascular invasion, and perineural invasion was obtained from patient records. The differentiation grade and TNM stage of OSCC were determined according to the seventh edition of the AJCC Cancer Staging Manual. The clinical diagnostic criteria of oral potentially malignant lesions were based on the WHO Collaborating Centre for Oral Cancer and Precancer in the UK [24].

Overall survival was defined as the period from the time of initial diagnosis (by biopsy or surgery) to death or last follow-up. Information obtained over the follow-up period was deleted from the analysis. Diseasefree survival was evaluated as the period from follow-up to recurrence.

Thirty-one freshly frozen OSCC tissues, 31 adjacent normal oral tissues, and 31 oral potentially malignant lesion tissues, all from the Affiliated Hospital of Stomatology, Nanjing Medical University, were also investigated. Tissue specimens obtained were stored in liquid nitrogen 10 min after surgery or biopsy. Patient clinical data were recorded in detail. The pathological diagnosis of OSCC and oral potentially malignant lesions were consistent with the pathological characteristics and was confirmed by two experienced pathologists at Nanjing Medical University.

This study was approved by the Research Human Ethics Committee of Nanjing Medical University (approval number: 201801154-1). All participants signed informed consent.

2.2. Quantitative real-time polymerase chain reaction (qRT-PCR)

To evaluate TROP2 mRNA expression levels, qRT-PCR was performed in 31 human OSCC tissues, 31 adjacent normal oral tissues, and 31 oral potentially malignant lesion tissues. All tissues samples were stored at −80 °C before use. The samples were ground in a ceramic grinder with liquid nitrogen. Using Trizol reagent (Invitrogen, Carlsbad, CA, USA), total RNA was isolated from the tissue samples according to the manufacturer's protocol. RNA was reverse-transcribed into cDNA sing the PrimeScript II 1 st Strand cDNA Synthesis Kit (Takara, GlenBurnie, MD, USA). The qRT-PCR amplified reactions were performed in 96-well plates on an ABI PRISM 7500 H T Sequence Detection System (Applied Biosystems, Foster City, CA. USA) with TROP2 primer (Genescript. Nanjing. China) and *GAPDH* primer as a control, as follows:

TROP2 forward, 5′-TGTCCTGATGTGATATGTCTGAG-3′ and reverse, 5′-GGGTGAGAGTGGGTTGGG-3′; *GAPDH* forward, 5′-GGAGCG AGATCCCTCCAAAAT-3′, reverse, 5′-GGCTGTTGTCATACTTCTCA TGG-3′.

The reaction conditions included 2 µl cDNA template, 10 µl of 2 × SYBR Green PCR Master Mixtures (Applied Biosystems), and 20 nmol/L of each primer in a total reactive volume of 20 µl. In subsequent qRT-PCR cycle conditions, the initial annealing temperature (to allow AmpErase-UNG activity) was set as 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 58 °C for 1 min. Each experiment was conducted at least three times.

TROP2 mRNA expression in each sample was normalized to *GAPDH*