Original Article

Expression of TLR1 in tongue squamous cell carcinoma and adjacent tissue

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Abstract: Toll-like receptors (TLRs) play a pivotal role in tumor progression. However, some of the underlying pathological mechanisms have been remain to be elucidated. Thus, in this study, we observe the distribution and expression of TLR1 (Toll like receptor, TLR1) in cancerous and adjacent tongue squamous cell carcinoma tissue, and explore the role of TLR1 in the pathogenesis of tongue squamous cell carcinoma. RT-PCR analysis showed that TLR1 had decreased expression in tongue squamous cell carcinoma specimens than that in matched adjacent normal tissues (P < 0.01). Western blot analysis further supported the similar results in protein expression of TLR1 in the cancerous and adjacent tissues (P < 0.01). The expression levels of TLR1 closely related of the clinical parameters of deeper tumor invasion, survival. Immunohistochemistry results showed TLR1 was mainly expressed in tongue normal squamous cell and vascular endothelial cell of duct, and weak expression in squamous cell carcinoma tissues. The immunofluorescence staining further revealed that TLR1 was co-location with the endothelial cell of blood vessels marker CD31, not co-location with the smooth muscle aorta cells marker α-SAM. The high expression of TLR1 in tongue carcinoma were significantly decreased closely associated with clinical progression, indicating that TLR1 plays a role in the developed process of tongue squamous cell carcinoma; it may be as an important prognostic indicator in clinical judgment.

Keywords: Toll-like receptors, TLR1, tongue squamous cell carcinoma, expression

Introduction

Oral squamous cell carcinoma (OSCC) is the most common of the oral and maxillofacial cancers, and 410,000 new OSCC patients are diagnosed every year, accounting for 1-5% of all cancers [1, 2]. OSCC is typically found in males between 40-50 years of age and is associated with various complications and nerve damage following surgical resection or radiation therapy [3, 4]. The 5-year survival rate for tongue cancer is approximately 50%, with no significant improvements in the survival rate and is associated with significant morbidity [5, 6]. Therefore, there is an urgent need to search for novel markers for OSCC and identify potential applications of gene therapy in OSCC treatment. To more accurately treat the disease, the underlying mechanisms responsible for its aggressiveness and new predictive markers of the disease are needed. In the last several decades, although multimodality therapy has improved the prognosis of patients, the survival rate of OSCC patients has remained unsatisfactory.

During the last decade, significant evidence has suggested that inflammation plays an important role in tumorigenesis, and microenvironment-derived signals are important constituents in all tumors [8]. The mediators and effectors of inflammation are essential components of the local environment of tumors, and the inflammatory conditions may be present before the malignant change occurs in some types of cancer [9]. Conversely, oncogenic changes have been shown to induce an inflammatory micro-
environment and further promote the development of tumors in some types of cancer [9]. As a result, the tumor microenvironment and tumor-associated inflammation mimic the response of innate immune cells and adaptive immune cells [8]. Toll-like receptors (TLRs) are classical signals that play a key role in the innate immune system. TLRs belong to a class of pattern-recognition receptors that play an important role in the host defense against pathogens by recognizing a wide variety of pathogen-associated molecular patterns (PAMPs) [10]. TLRs drive the inflammatory response and control cell proliferation and survival by stimulating immune cells and promoting integrated processes of inflammatory responses and tissue repair.

A recent study revealed that TLR families, such as TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9, are expressed in various head and neck squamous cell carcinomas in vivo and in vitro [2, 11]. Further study indicated TLR2, TLR4 and TLR9 are associated with the invasive potential of early-stage head and neck squamous cell carcinoma. Furthermore, TLR9 expression correlates with advanced tumor size, and TLR5 expression is correlated to worse survival [2]. Another study demonstrated that systemic injection of a TLR1/2 agonist increased adoptive antigen-specific T cell therapy in glioblastoma-bearing mice [12] and also induced tumor regression by reciprocal modulation of effector and regulatory T cells [13]. Previous studies have demonstrated that TLR1 and TLR2 cooperate in the recognition of the lipopeptides [14, 15]. These results also suggest that TLR1 may also play an important role in tumors. Therefore, this study evaluated the expression and distribution of TLR1 in tongue squamous cell carcinoma and correlated the expression of these proteins with various clinico-pathological variables.

Materials and methods

Patient samples

All samples were collected from 60 patients undergoing glossectomy for tongue squamous cell carcinoma (during the period January 2013 to January 2015) in the Department of Surgery at Stomatological Hospital of Chongqing Medical University, China. In this study, all patients provided written informed consent prior to participation. Histopathological slides were reviewed by a senior pathologist to confirm the diagnosis and analysis. Among the analyzed individuals, the mean age was 57 years, and the patients comprised 40 men (67%) and 20 women (23%). Moreover, the collection of clinical data included the degree of differentiation and clinical stages. According to the TNM staging system [16] and clinical staging of tongue squamous cell carcinoma, all cases were divided into 10 stage I-II cases and 50 stage III-IV cases. This study was approved by the Research Ethics Committee of Chongqing Medical University.

Sample processing

For genetic and protein analysis, tissues samples were preserved in liquid nitrogen after the samples were removed. For immunohistochemical staining and immunofluorescent staining, the tissue samples were fixed in a solution of 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 24 h, and the samples were embedded with paraffin and cut into 4-5-μm sections.

Hematoxylin and eosin (HE) staining

The OSCC tissues were obtained from the surgery and fixed prior to dehydration in ethanol and paraffin-embedded. Following the embedding of the tissues in paraffin wax, the samples were cut into 5-μm sections, which were deparaffinized in water, stained with hematoxylin-eosin (HE) and sealed with neutral gum. The pathological changes of the OSCC were evaluated by two pathologists who had no prior information with respect to the treatment.

Isolation of RNA and RT-PCR

Total RNA was isolated from the tongue squamous cell carcinoma specimens and the matched adjacent tissues using Tiangen RNA kit (Tiangen Reagents Ltd, Beijing, China), according to the manufacturer’s instructions. The RNA was further checked with the Nanodrop ND-100 analytical instrument (Thermo Fisher Scientific Inc., USA), and the OD260/OD280 ratio of the total RNA extracted from tissue samples ranged from 1.8 to 2.0. cDNA was synthesized from extracted RNA using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara Reagents, Dalian, China) according to the manufacturer’s instructions. The polymerase chain
reaction used the 2 × PCR Reagent (Tiangen Reagents Ltd, Beijing, China). The following primers were used: GAPDH (Forward: AATCC-CATACCATCTTCC; Reverse: CATCACGCCACAGTTTCC) and TLR1 (Forward: CAGGTTTAGACGCCTTTT; Reverse: AGTCACGACCAACACC). The PCR cycling parameters were as follows: pre-denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 40 sec, and extension at 72°C for 5 min, performed using the ABI Model 7300 PCR machine. PCR products (6 μL) of 328 bp (TLR1) and 382 bp (GAPDH) were analyzed electrophoretically using 2.5% agarose gel electrophoresis and viewed under ultraviolet (UV) light. The mRNA expression of TLR1 was normalized by the expression of GAPDH.

Western blot

The tongue squamous cell carcinoma specimens and the matched adjacent tissues were harvested and lysed in lysis buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, sodium pyrophosphate, β-glycerophosphate, EDTA, Na3VO4, leupeptin and protease inhibitors (Roche Molecular Biochemicals). Aliquots of tissues lysates were resolved by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, USA). The membranes were blocked in 5% (w/v) bovine serum albumin (BSA) in TBST buffer for 2 h at room temperature. Primary antibody (1:1000; TLR1, Abcam, USA) and β-actin (1:1000; Santa Cruz, USA) were diluted in TBST buffer and incubated with membranes overnight at 4°C. HRP-conjugated secondary antibodies for TLR1 and HRP-conjugated anti-mouse antibodies for β-actin diluted in TBST buffer and incubated with membranes overnight at 4°C. HRP-conjugated anti-rabbit antibodies for TLR1 and HRP-conjugated anti-mouse antibodies for β-actin diluted in TBST buffer were used to detect the primary antibody. Membranes were incubated with the secondary antibody for 2 h at room temperature. A luminol-based enhanced chemiluminescence (Fisher Scientific, UK, Loughborough, UK) method was used to visualize the immunoreactive protein bands. The protein expression of TLR1 was normalized by the expression of GAPDH.

Immunohistochemistry and immunofluorescence staining

The section were dewaxed with xylene and hydrated with graded alcohol. Antigen retrieval was performed by incubation in a 95°C 0.01 M citrate buffer (pH = 6.0) for 10 minutes followed by 30 minutes of cooling at room temperature. Sections were placed in 3% hydrogen peroxide (H2O2) for 5 min for the inhibition of endogenous peroxide activity. After three washes with PBS buffer, the sections were incubated with 5% donkey serum in 0.1% Triton X-100 in phosphate-buffer saline (PBS). Subsequently, the sections were incubated with the polyclonal antibody specific to TLR1 (1:100; Abcam, USA) at 4°C overnight. The overnight incubation was followed by three washes with PBS buffer, and the sections were incubated with biotinylated secondary antibody for 30 min and peroxidase-labeled streptavidin (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd, Beijing, China) for 25 minutes. The tissues were then stained with 3,3-diaminobenzidine solution for 2 minutes and sealed. Slides were again counterstained with Mayer’s hematoxylin. The primary antibody was omitted as a negative control and was replaced with PBS.

For immunofluorescence staining, after antigen retrieval, the sections were incubated with 5% donkey serum in the 0.1% Triton X-100 in phosphate buffer saline (PBS). Sections were incubated with the polyclonal antibody specific to TLR1 (1:100; Abcam, USA), α-SAM (1:100; Abcam, USA), or CD31 (1:100; Abcam, USA) at 4°C overnight. After washing three times with PBS, sections were incubated with donkey anti-rabbit secondary antibodies conjugated with DyLight™ 647 (Thermo Scientific) and donkey anti-mouse secondary antibodies conjugated with DyLight™ 488 (Thermo Scientific). Nuclei were stained with DAPI.

The staining intensity was graded using the following standard based upon the previous publication [17]: 0 for weakly stained cells, 1 for moderate yellow cells, 2 for cells stained orange, and 3 for cells stained brown. The percentage of stained cells was 0 for ≤ 25% staining, 1 for 25-50%, 2 for 50%, 3 for 51-75%, and 4 for more than 75% staining. The staining scores of 0-3 points were considered low expression, and scores of 2-4 points were considered high expression [17].

Statistical analysis

Statistical analysis was performed using SPSS Statistics 18.0 software. The data are presented as the mean ± SD. Student’s t-test was used
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Results

Morphological features of carcinoma tissue and adjacent normal epithelia tissue

Representative histological images of tongue squamous cell carcinoma tissue revealed more than 70 ± 14% tumor cells and included many more keratin pearls and greater amounts of keratin in intercellular bridges. High-power fields revealed nuclear fission, multinucleated giant cells, and polymorphic nuclei (Figure 1A-D). The representative images were microdissected from relatively large areas of tongue squamous cell carcinoma tissue and adjacent normal epithelia/mucosa tissue. The adjacent normal epithelial tissue did not show significant contamination of stromal cells, infiltrating inflammatory cells, vascular components, muscular components or necrotic cells [18] (Figure 1A-D).

TLR1 mRNA and protein expression were significantly decreased in human tongue squamous cell carcinoma tissue

We first investigated the mRNA expression of TLR1 in 60 human tongue squamous cell carcinoma tissue samples and adjacent tissue samples using RT-PCR. The amplified products of the target mRNA for TLR1 (approximately 328 bp) and GAPDH mRNA (approximately 382 bp) were observed electrophoretically using 2.5% agarose gel electrophoresis. Products from the amplification of the target mRNA were consistent with the TLR1 gene sequence. As indicated in Figure 1, we observed the 328-bp band of TLR1 and the 382-bp band of GAPDH. The results revealed that the expression of TLR1 was significantly lower in tongue squamous cell carcinoma tissues compared with that in non-cancerous tissues (P < 0.05; Figure 1).

Next, we investigated the expression of the TLR1 protein by western blot. From the blot gel, we observed the target protein band for TLR1 (approximately 89 kDa) and GAPDH (approximately 37 kDa), and we were able to detect the protein band for TLR1 in both tongue squamous cell carcinoma tissue and adjacent tissue (Figure 2). The protein gel blot assay revealed that the expression of TLR1 was significantly lower in tongue squamous cell carcinoma tis-
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Figure 2. mRNA expression of TLR1 in tongue squamous cell carcinoma tissue and adjacent tissue. The top panel presents the graph and indicates the mean value of TLR1 in the tongue squamous cell carcinoma tissue and adjacent tissue. The lower panel presents the representative band of TLR1 mRNA expression in the tongue squamous cell carcinoma tissue and adjacent tissue. The TLR1/GAPDH mRNA ratio indicates that TLR1 was significantly decreased in the tongue squamous cell carcinoma tissue compared with the adjacent tissue. n = 8 per group, **P < 0.01 vs. the tongue squamous cell carcinoma group. M, DNA marker.

Figure 3. Protein expression of TLR1 in tongue squamous cell carcinoma tissue and adjacent tissue. Top panel presents the western blot analysis of TLR1 in the tongue squamous cell carcinoma tissue and adjacent tissue. The lower panel presents the representative band of TLR1 protein expression in the tongue squamous cell carcinoma tissue and adjacent tissue. Quantification of western blots for TLR1 demonstrates that TLR1 was significantly decreased in tongue squamous cell carcinoma tissue compared with the adjacent tissue. n = 8 per group, **P < 0.01 vs. the tongue squamous cell carcinoma group.

All cancer specimens exhibited weak staining for TLR1, whereas normal squamous epithelium exhibited negative to positive staining in the cytoplasm. The clinicopathologic features and expression findings are presented in Table 1. No significant relationship was found between TLR1 expression and age, and the expression of TLR1 did not differ significantly between 40 cases of male patients and the 20 cases female patients. The expression of TLR1 did not differ between patients older than 40 years and those younger than 40 years. Statistical analysis revealed that TLR1 expression was positively correlated with pathological differentiation and clinical stages in tongue squamous cell carcinoma tissue. A summary of expression of the proteins in various histological categories of tongue squamous cell carcinoma is presented in Table 1.

TLR1 was localized in the ducts of adjacent normal tissue

Having characterized the gene and protein expression of TLR1 in tongue squamous cell carcinoma tissue and adjacent tissue and having determined that TLR1 was significantly decreased in tongue squamous cell carcinoma tissue compared with adjacent tissue, we next observed the distribution of TLR1 in paraffin sections. The representative immunohistochemical features of the proteins are presented in Figure 4. In cases of both high and low differentiation, most carcinoma samples exhibited weak expression of TLR1. In contrast, most of the normal epithelial samples exhibited positive expression of TLR1 (97%). In the normal epithelium samples, TLR1 was found to be primarily localized to the cyto-

Correlations between TLR1 expression and clinicopathologic parameters

All patients (n = 60) were divided into two groups according to the different parameters.
plasm or nucleus (Figure 4A-D), but TLR1 was largely localized to the cell membrane in tongue squamous cell carcinoma tissue (Figure 4E, 4F). In the normal epithelium samples, all of the TLR1 expression was distributed in the ducts or blood vessels in adjacent normal epithelia/mucosa tissue (Figure 4A-D).

From the expression characteristics of TLR1 in adjacent tissue, we determined that TLR1 was distributed in the ducts or blood vessels. Therefore, we further confirmed the characteristics of TLR1 location in adjacent tissue through the use of immunofluorescence. TLR1 was not co-expressed with α-SMA (alpha-smooth muscle actin), a representative marker of smooth muscle actin cells (Figure 5A-D). However, TLR1 was strongly co-expressed with the CD31, a representative marker of vascular endothelial cells (Figure 5E, 5F). The results indicate that the blood vessel endothelium of the surrounding tissue can strongly secrete TLR1 (Figure 5).

Discussion

Emerging evidence now implicates TLRs in inflammation-associated cancers. Therefore, understanding the roles of TLRs in tumor biology may pave the way for the discovery of novel therapeutic targets in cancer therapy. In this study, significantly decreased mRNA and protein levels of TLR1 were observed in tongue squamous cell carcinoma tissue relative to adjacent tissue, and the decreased expression was closely associated with the clinical progression of tongue squamous cell carcinoma. The morphological data further demonstrated that TLR1 was primarily expressed in the vascular endothelial cells within the adjacent normal tissue.

The mammalian Toll-like receptor (TLR) family consists of 13 members, and each recognizes particular microbial compositions, called pathogen-associated molecular patterns (PAMPs). The recognition of TLR-dependent PAMPs induces innate immune system activation, which later leads to the activation of the antigen-specific adaptive immunity system, resulting in increased expression of the inflammatory cascade via the MyD88-dependent pathway and TRIF-dependent pathway [19]. Originally, research into TLRs focused on their association with microbial pathogens, but recent work suggests that TLRs also recognize damage/danger-associated molecular patterns (DAMPs), which are released from damaged and dying cells, including heat shock proteins (HSP), nucleic acids, and high-motility group box-1 protein (HMGB1) [20, 21]. Tumor cells can activate TLRs through DAMPs. There is strong evidence that activated TLRs cells can promote the supervision and removal of newly developed tumors [22, 23]. Recent work indicates that functional TLRs play an important role in tumor biology. The TLR-activated tumor cells promote proliferation of tumor cells and also antagonize cell apoptosis, simultaneously improving the invasive ability of tumor cells through the acquisition of metalloproteases and integrin [10]. TLR signaling can further activate tumor cells to induce the synthetic and immunosuppressive actions of inflammation, leading to immune escape by enhancing the resistance of tumor cells to anti-cytotoxic leukocyte attack [24].

Preliminary evidence suggests that chronic inflammation may play an important role in tumorigenesis and tumor progression [25]. In humans, TLR activation is an integral component of the inflammatory response to pathophysiological stimuli. Chronic activation of TLRs is associated with the aggressiveness of various cancers and with poor prognosis [26]. Substantial evidence indicates that TLR4 activation has been detected on tumor cells, and

Table 1. Summary of the statistical significance of the various variables for tumor tissues

<table>
<thead>
<tr>
<th>Pathological features</th>
<th>n</th>
<th>TLR1 expression</th>
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<th>P</th>
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<tbody>
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<td>Sex</td>
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<tr>
<td>Male</td>
<td>40</td>
<td>35 (87.5)</td>
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<tr>
<td>Female</td>
<td>20</td>
<td>18 (95.0)</td>
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<tr>
<td>Age</td>
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<td></td>
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<td>0.566</td>
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<tr>
<td>≥ 40</td>
<td>54</td>
<td>49 (90.7)</td>
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<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>6</td>
<td>5 (83.3)</td>
<td></td>
<td></td>
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<tr>
<td>Differentiation</td>
<td></td>
<td></td>
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<td>0.911</td>
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<tr>
<td>High</td>
<td>49</td>
<td>44 (88.8)</td>
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<tr>
<td>Low</td>
<td>11</td>
<td>10 (90.9)</td>
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<td>Tumor size (T)</td>
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<tr>
<td>T1-T2</td>
<td>8</td>
<td>6 (75.0)</td>
<td>2.308</td>
<td>0.129</td>
</tr>
<tr>
<td>T3-T4</td>
<td>52</td>
<td>48 (92.3)</td>
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<td>TNM staging</td>
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<td></td>
<td>21.33</td>
<td>0.000</td>
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<tr>
<td>I-II</td>
<td>10</td>
<td>5 (50.0)</td>
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<tr>
<td>III-IV</td>
<td>50</td>
<td>49 (98.0)</td>
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TLR1 expression in tongue squamous cell carcinoma

this activation can induce chronic inflammation and further increase the tumor growth [27]. Another study demonstrated that TLR9 activation promotes tumor cell proliferation of prostate cancer [28]. In contrast, the stimulation of TLR9 on lung cancer cells can sensitize tumor cells to apoptosis and promote tumor growth arrest [29]. These data indicate context-dependent roles for different TLRs in tumor progression. Through our observations at the gene and protein level, we determined that the expression of TLR1 in tongue squamous cell carcinoma is significantly lower than in the surrounding tissue, indicating that the reduced expression of TLR1 may play a role in the development of carcinoma. TLR2 is known to be expressed in oral squamous cell carcinoma tissue and plays an important role in Treg expansion and suppressive capacity [30, 31]. Other research determined that TLR3 is expressed in head and neck cancer cell lines and oral squamous cell carcinoma tissue, and the over-expression of TLR3 is closely related to the activity of NF-κB. Conversely, inhibiting the expression of TLR3 decreases the expression of the NF-κB-regulated oncogene c-myc and cell proliferation, and stimulating the expression of TLR3 significantly increases the invasiveness capacity and aggressiveness of tumor cells [32, 33]. Another study found that TLR4 is expressed in oral squamous cell carcinoma cell lines, and the expression level is related to tumor differentiation [34]. TLR4 is highly expressed in well-differentiated carcinomas, and the expression level of TLR4 is closely associated with the degree of oral epithelial dysplasia and the distribution of cancer cells [35]. Another research study indicated significantly elevated expression of TLR5 in tongue cancer relative to surrounding tissue, and the prognosis of tumors expressing high levels of TLR5 is typically poor [36]. Similarly, the expression of TLR9 in tongue cancer is much more elevated than in the surrounding tissue, and the increased expression of TLR9 correlates with the proliferation of oral squamous cell carcinoma cells and tumor invasiveness [37, 38]. Furthermore, the correlation with clinical parameters indicates that the change in TLR1 immunocompetence in tumor tissue is related to clinical progress and prognosis, such as the TNM staging of tumor cells. Furthermore, the mode of invasion and the malignancy grading system is known to play a key role in tumorigenesis [39]. In this study, we

Figure 4. Immunohistochemical staining of TLR1 protein in tongue squamous cell carcinoma tissue and adjacent tissue. (A, B) Representative images of TLR1 immunostaining in adjacent normal epithelial tissue and tongue squamous cell carcinoma tissues (A, B). Representative images of TLR1 in adjacent normal duct tissue and tongue squamous cell carcinoma (B, C). Representative images of TLR1 in tongue squamous cell carcinoma tissue (E, F). High-magnification (A, C, E) and low-magnification (B, D, F) views of cells stained with TLR1 in the adjacent normal epithelial tissue adjacent to tongue squamous cell carcinoma. Arrows indicate TLR1 expression in positive cells. (A, C, E) × 400; (B, D, F) × 1200; bars = 20 μm (A, C, E), bars = 80 μm (B, D, F).
also determined that stage III or IV OSCC was correlated significantly with late cervical metastasis. Our study demonstrated that the expression of TLR1 was low in tumor tissue and high in surrounding tissue, indicating that TLR1 may play an important role in maintaining the stability of the human tongue squamous cell carcinoma internal environment and promoting tumor immune supervision, also indicating that TLR1 may serve as an important predictive factor of tongue squamous cell carcinoma.

The internal and external environment of tumor cells, tumor cell differentiation, surrounding immune and inflammatory cells, and vascular endothelial cells play important roles in the development and progression of tumors [40]. The tumor microenvironment is well established to play a crucial role in resistance to radiation and chemotherapy and plays an important role in the initiation, progression, invasion and metastasis of tumor tissue [41]. Our morphology results indicate that TLR1 is largely distribute in the cytoplasm and the nucleus of the surrounding normal tongue epithelial cells; in contrast, TLR1 is only weakly expressed in the membrane of tumor cells in tongue squamous cell carcinoma. These results suggested that TLR1 regulates tumor cells via different signaling pathways in the cell membrane, cytoplasm and nucleus. The study indicated that the cell signaling transmits signals to the cell nucleus and may interact with other signaling factor related to intracellular growth and differentiation, activating special signaling pathways [42]. Accordingly, we assume that the tumor may influence TLR1 function to modulate the signal transduction pathway of differentiating cells, inducing the uncontrolled growth of cells, ultimately promoting the development and progression of tumorigenesis. TLR1, TLR2, TLR4, TLR5 and TLR10 are usually expressed on the surface of healthy immune and epithelial cells; nevertheless TLR3, TLR7, TLR8, and TLR9 are typically expressed on the surface of endosomes, lysosomes and the endoplasmic reticulum [43, 44]. The expression pattern of TLRs differs within tumor tissue and the surrounding normal tissue. This feature may suggest that the activation of TLRs depend different signaling pathways, inducing the tumor immune response. However, the effect of the TLR immune response is ambiguous and not fully understood at present.

TLRs can regulate the immune system by up-regulating immunosuppressive protein factors such as vascular endothelial growth factor (VEGF) and releasing growth factors in the tumor microenvironment [45]. It is now gener-
ally accepted that tumor angiogenesis is necessary for the growth of primary neoplastic tumor and the development of metastasis [46]. Some studies have indicated that tumor angiogenesis is related to tumor progression and malignancy [47]. The VEGF induces the proliferation, differentiation, and migration of vascular endothelial cells and increases the permeability of the capillaries, further enhancing the survival of endothelial cells by preventing their apoptosis [46]. Our immunofluorescence staining demonstrated that TLR1 was mostly expressed in vascular endothelial cells positive for CD31, indicating that TLR1 is released from vascular endothelial cells downstream of the cell immune response and may play an important role in the pathogenesis of tongue carcinoma. The results also determined that TLR1 is not expressed in the vascular smooth muscle cells of the tumor, revealing that the regulatory function of TLR1 may be affected in the progression of tongue squamous cell carcinoma to promote the invasion and progression of the tumor. Morphological evidence further demonstrated that tumor cells can inhibit the activation of TLR1 in tumors, indicating that TLR1 plays a positive role in inhibiting the growth of tumors. The elevated expression of TLR1 in normal tongue epithelial tissue may inhibit the growth of the tumor.

In conclusion, our research described the expression and distribution of TLR1 in tongue squamous cell carcinoma and the relationship between TLR1 and clinical pathological features. Our study illustrates that TLR1 plays a positive role in the development and progression of tongue squamous cell carcinoma. However, the regulatory mechanism of TLR1 in tongue squamous cell carcinoma requires additional research; these findings will play an important role in revealing the regulation of TLR1 in tumor pathology and targeting tumor TLR signaling pathways.

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Disclosure of conflict of interest

None.

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