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Differential expression of organic cation transporter 3 in oral submucous fibrosis–associated buccal squamous cell carcinoma

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Objective. The aim of this study was to examine the expression of organic cation transporter 3 (OCT3) in patients with oral submucous fibrosis (OSF)–associated buccal squamous cell carcinoma (BSCC) and to explore its clinical significance.

Study Design. A total of 56 tissue specimens were collected from patients, among which there were 13 specimens with normal buccal mucosa (NBM), 13 with oral submucous fibrosis (OSF), 10 with OSF-associated BSCC (BSCC-OSF), 10 with well-differentiated BSCC (BSCC-I), and 10 with poorly to moderately differentiated BSCC (BSCC-II+III), based on pathologic examination. The expression of OCT3 was detected by using immunohistochemistry and real-time quantitative reverse transcription polymerase chain reaction.

Results. There was a significant difference in both the protein and mRNA expression levels of OCT3 among the NBM, OSF, BSCC-OSF, BSCC-I, and BSCC-II+III groups (protein: $F = 82.45$ [$P < .0001$]; mRNA: $F = 50.69$ [$P < .0001$]). The expression of OCT3 from NBM to OSF to BSCC-OSF was gradually upregulated. In addition, as BSCC became better differentiated, the expression of OCT3 increased.

Conclusions. The expression of OCT3 was associated with OSF progression and the differentiation of BSCC. OCT3 expression may serve as a molecular marker for the prevention and early diagnosis of OSF and BSCC. (Oral Surg Oral Med Oral Pathol Oral Radiol 2018;■■:■■–■■)

Buccal squamous cell carcinoma (BSCC) is one of the most common types of oral cancer and the sixth most common malignant tumor worldwide.[1] The 5-year survival rate is approximately 50% because of frequent local relapse and distant metastasis despite modern medical treatment.[2] Thus, prevention and early diagnosis are of particular importance in combatting BSCC. As an oral precancerous status listed by the World Health Organization (WHO), oral submucous fibrosis (OSF) is a chronic and insidious lesion of the oral mucosa.[3] The habit of chewing areca nuts is one of the major etiologic factors for OSF, which results in the high prevalence of OSF in South and Southeast Asian countries.[4,5] The early stage of OSF is characterized by stomatitis, which presents as an inflammatory reaction at the juxtaepithelial region, whereas in moderate to advanced stages, it presents as a white fibrosis band and limitation of mouth opening caused by collagen dysregulation at the lamina propria and the deeper connective tissues with associated epithelial atrophy.[3,6]

Patients with OSF have a high risk, ranging from 2.3% to 7.6%, for oral cancer.[7-9] Among oral precancerous lesions or status, the malignant transformation rate of OSF ranks second, only after that of oral leukoplakia (0%–36.4%).[10] In contrast to the well-understood carcinogenesis of oral leukoplakia, the molecular mechanism of the OSF malignant transformation process remains unclear. Some authors believe that collagen accumulation in the submucosa and consequent vascular reduction lead to tissue hypoxia, which is a cancer-inducing factor.[11] Arecoline, which is abundant in the areca nut, was found to have cytotoxicity and genotoxicity to the oral mucous epithelium and may cause progression to precancerous lesions.[12] Multiple oncogenes and related pathways, such as survivin, cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMPs), P53, and Wnt signaling

Statement of Clinical Relevance

The expression of organic cation transporter 3 (OCT3) in oral submucous fibrosis (OSF)–associated buccal squamous cell carcinoma (BSCC) remains unclear. Here, we have provided a description of the differential expression of OCT3 in OSF and BSCC, which may serve as a molecular marker for the prevention and early diagnosis of OSF and BSCC.

Conflict of interest: The authors declare that they have no financial, personal, or any other conflicts of interest that influenced, or could be perceived to have influenced, this work.

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pathways, have been investigated for their role in the transition from OSF to BSCC-OSF.[13,14] In 1953, Slaughter first proposed the "field cancerization" theory, which suggested that after repeated carcinogenic exposures, the epithelium of the oral mucosa has an increased risk of developing precancerous lesions because of multiple genetic abnormalities.[15,16] Many patients with OSF and BSCC-OSF have the habit of chewing areca nuts. Even if they stop the habit later, the cancerous field with genetic abnormalities can continue to exist.[7] This theory may explain the high malignant transformation rate of OSF. Moreover, although the frequent relapse and recurrence of BSCC-OSF may sometimes result from incomplete incision, the transformation from additional diffuse cancerous fields could be critical. Because these different cancerous fields cannot be completely removed surgically, strategies to prevent their transformation to cancer, such as the use of pharmacologic agents, should be explored.

Organic cation transporter 3 (OCT3) is an important membrane transporter, which plays a role in cellular metabolic homeostasis by transporting endogenous and exogenous cation compounds across the membrane. It belongs to the *SLC22* family, which contains the 3 members of organic cation transporters OCT1 (*SLC22 A1*), OCT2 (*SLC22 A2*), and OCT3 (*SLC22 A3*). In contrast to human OCT1 and OCT2, which are highly expressed in the liver and kidneys, respectively, human OCT3 is widely expressed in a variety of tissues.[17] The polymorphisms and expression levels of OCTs have been reported to contribute to drug sensitivity and resistance, as well as various nervous diseases.[18] For example, the sensitivity of certain drugs, such as oxaliplatin, is related to differential expression levels of OCT3 in tumor tissues.[19] Interestingly, in addition to drug response, the expression of OCT3 seems to be related to risk, malignancy degree, and prognosis of cancer.[20] OCT3 has been reported to be upregulated in oral, liver, rectal, and cervical cancers but downregulated in prostate cancer and is associated with the degree of cancer malignancy.[21-23]

The expression of OCT3 in OSF and BSCC is unknown. In a pilot attempt to explore the potential of OCT3 expression as a biomarker for prevention and early diagnosis of OSF and BSCC, we detected and compared the expression of OCT3 in normal buccal mucosa (NBM), OSF, OSF-associated BSCC (BSCC-OSF), well-differentiated BSCC (BSCC-I) and poorly to moderately differentiated BSCC (BSCC-II+III), by using immunohistochemistry (IHC) and real-time quantitative polymerase chain reaction (RT-qPCR). The results collected from the different stages of buccal mucosa for BSCC may provide an insight into OSF carcinogenesis and have implications in choosing OSF and BSCC patients who might benefit from certain agents of OCT3 substrates.

MATERIALS AND METHODS

Patients and tissue collection

Fifty-six tissue specimens were collected from patients in the Department of Oral and Maxillofacial Surgery, Xiangya Hospital, Central South University from October 2014 to October 2016. There were 13 NBM, 13 OSF, 10 BSCC-OSF (Figure 1), 10 BSCC-I, and 10 BSCC-II+III cases. Detailed description of clinical and histopathologic characteristics is provided in Table I. NBM tissues were obtained from healthy volunteers. All enrolled patients with OSF or BSCC-OSF had the habit of areca nut chewing, whereas the NBM and BSCC cases did not have this habit. Each specimen was divided into 3 parts: 1 for pathologic review to confirm the diagnosis and the remaining 2 were respectively embedded in paraffin for IHC and immediately snap-frozen in liquid nitrogen at −80°C for RT-qPCR analysis. All clinical diagnoses and histologic evaluations were performed according to the WHO standard criteria.[24,25] All patients were informed about the aims of specimen collection, and signed written consent were obtained in accordance with the ethical guidelines of Xiangya Hospital, Central South University. Concealed allocation and blindness were applied during tissue collection and the experiments, as discussed below.

Immunohistochemistry

Tissue specimens were embedded in paraffin and cut into continuous sections with a thickness of 4 μm. Following deparaffinization, dehydration, and antigen retrieval, the sections were incubated with 1:200 diluted rat monoclonal antibodies against human OCT3 at 4°C overnight. They were then washed with phosphate-buffered saline and incubated at 37°C in a water bath for 2 hours after drop-adding the second antibody and washing with phosphate-buffered saline. After treatment with 3, 3

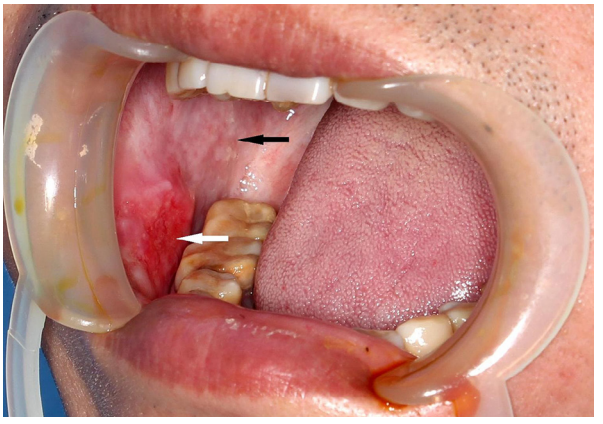

Fig. 1. OSF-associated BSCC (BSCC-OSF). The black arrow refers to OSF, and the white arrow refers to BSCC. *BSCC-OSF,* oral submucous fibrosis–associated buccal squamous cell carcinoma (BSCC); *OSF,* oral submucous fibrosis.