Second primary tumors and myeloperoxidase expression in buccal mucosal squamous cell carcinoma

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Objective. The present study investigated the relationship between the expression of manganese superoxide dismutase (MnSOD), catalase, glutathione peroxidase (GP*x*), and myeloperoxidase (MPO) in buccal mucosal squamous cell carcinoma (SCC), and the risk of second primary tumors (SPTs).

Study design. Immunohistochemistry was performed to examine the expression of MnSOD, GP*x*, catalase, and MPO in tissue microarray slides of 173 male patients with buccal mucosal SCC who had undergone potentially curative resections.

Results. Forty-five (26.01%) patients developed SPTs. The prevalent subsites of SPTs were buccal mucosa (48.89%), tongue (13.33%), and lip (11.11%). High expression level of MPO was correlated with an increased risk of SPTs, even after adjustment for development of clinicopathologic parameters (high vs. low expression, adjusted hazard ratio = 3.89; 95% confidence interval, 1.33-11.41; $P = .013$).

Conclusions. SPTs are common in male buccal mucosal SCC patients. Higher MPO expression in buccal mucosal SCC is a risk factor for SPTs. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:464-473)

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Oral and pharyngeal cancer is one of the most common cancers, listed as the sixth leading cancer worldwide.[1] The disease is difficult to control after treatment because of its high propensity for second primary tumors (SPTs).[2] A population-based study of SPTs from the Surveillance, Epidemiology, and End Results (SEER) Program (National Cancer Institute, Bethesda, MD), between 1974 and 2003 in the United States, reported that oral and pharyngeal cancer showed the second most frequent occurrences of multiple primary tumors (9697/65,210 = 14.9%).[3] Squamous cell carcinoma (SCC) of the buccal mucosa is the most common form of oral and pharyngeal cancer in South and Southeast Asia, including Taiwan.[4,5] Betel quid chewing, cigarette smoking, and alcohol drinking are the major risk factors for the high incidence of buccal mucosal SCC in Taiwan.[6,7] In addition, SCC originating in the buccal mucosa acts more aggressively than SCC

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Statement of Clinical Relevance

Second primary tumors (SPTs) are quite common (26%) in male buccal mucosal squamous cell carcinoma (SCC) patients. Myeloperoxidase expression can be used as a potential biomarker of susceptibility to SPTs in buccal mucosal SCC patients.

originating in other subsites of the oral cavity.[8] We chose to evaluate the status of SPTs and risk factors of SPTs for buccal mucosal SCC.

SPTs in oral cancer occur with a probability of 6%-27%,[2,3,9-23] depending on the duration of the patient's follow-up,[24] statistical methodology, and definition of SPTs. The risk factors related to environmental and clinicopathologic parameters for SPTs among oral cancers have been extensively investigated. For example, certain studies have reported that cigarette smoking,[25-28] alcohol drinking,[25-28] index tumor location at specific subsites of the oral cavity (e.g., the lower part of the mouth or non-tongue area),[9,29] lower tumor stage,[16,25] and lower nodal stage[9,16] are risk factors for SPTs. In addition, Scholes et al.'s genetic study on SPTs of oral SCC (index cancer) using microsatellite markers demonstrated that 40% of SPTs are of independent origin, but 60% of SPTs have a common clonal origin as index cancers.[30,31] Tabor et al.'s study also supports that SPTs share some or even all genetic markers with the index tumor, indicating that both index tumors and SPT had arisen from a common clonal progenitor cell.[32,33] Therefore, the molecular characteristics of index tumors may be correlated with the occurrence of SPTs.

Reactive oxygen species (ROS) play a key role in human cancer development by causing DNA base changes, strand breaks, damage to tumor-suppressor genes, and enhanced expression of proto-oncogenes.[34,35] ROS has multiple detrimental effects upon the oral mucosa, including tumorigenesis and tumor progression. On the basis of the concept of "field cancerization," prolonged exposure to ROS generated during betel quid chewing, cigarette smoking, and alcohol drinking may lead to SPTs indicating independent transformation of multiple epithelial cells at several sites. The production and release of ROS occur under alkaline conditions (because of slaked lime supplemented in betel quid) during the auto-oxidation of betel quid polyphenols in the betel quid chewers' saliva.[36] In addition, arecoline, which is one of the main components of betel quid, causes depression of antioxidants (e.g., superoxide dismutase, catalase, glutathione peroxidase (GP*x*), and glutathione-*S*-transferase) that are known to neutralize ROS.[37] Each puff of cigarette smoke contains some ROS (e.g., superoxide, hydrogen peroxide, as well as hydroxyl and peroxyl radicals),[38,39] which consume more antioxidants during smoking. Alcohol metabolism by nicotinamide adenine dinucleotides (NADH) oxidation in the electron transport chain generates ROS that result in lipid peroxidation and protein adduct formation.[40]

Myeloperoxidase (MPO), an oxidant enzyme, produces ROS. Manganese superoxide dismutase (MnSOD), catalase, and GP*x* are members of the enzymatic antioxidant system that neutralize ROS. Our previous study evaluated the association of the levels of the above 4 proteins in tissue arrays and prognosis of patients with buccal mucosal SCC. We found that MnSOD and GP*x* were significant prognostic factors for favorable survival and the status of multiple primary tumors was associated with poor cancer-specific survival.[41] However, patients with "surgical margins not clear" and patients with "buccal mucosa not the index cancer" were also included in our previous study. Therefore, in this study, we aimed to evaluate the status of SPTs and identify whether the expression of MPO, MnSOD, catalase, and GP*x* in buccal mucosal SCC tissues is correlated with subsequent SPTs in patients with buccal mucosal SCC (index cancer) after surgical resection with clear margins.

MATERIALS AND METHODS

Patients and tissue specimens

The study protocol was approved by the Institutional Review Board of the Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan; IRB number: VGHKS97-CT2-08) and was in compliance with the ethical standards of the Declaration of Helsinki. Because of the limited number of female patients ($n = 4$), only 173 male patients were recruited in this study between 1993 and 2006. All were histologically confirmed to have buccal mucosal SCC (index cancer) that was treated by potentially curative resection and had clear margins ($\geqq$2 mm). The SPT data were collected from the time of operation to January 2012. The median follow-up period was 71.27 months (range 0.27-235.23 months). Patient follow-up included clinical examination by oral and maxillofacial surgeons, head and neck surgeons, or radiation oncologists. Additional radiographic and biopsy examinations were performed when a recurrence or an SPT was suspected. The definition of SPT was refined from the criteria established by Warren and Gates.[42] An SPT was defined as a new tumor of different histologic type, one of identical histologic type occurring >3 years after therapy of the primary tumor, or one separated from the initial primary tumor by >2 cm of clinically normal epithelium.[2,25,43,44] A metachronous SPT was defined as a malignancy diagnosed more than 6 months after the appearance of the index tumor. A synchronous SPT was defined as a malignancy diagnosed simultaneously or within 6 months after the appearance of the index tumor. Pathologic staging at initial presentation was performed according to guidelines of the 2002 American Joint Committee on Cancer system.

Tissue microarray construction

The detailed procedures in tissue microarray (TMA) construction were described in our previous study.[41]

Immunohistochemistry

Rabbit polyclonal anti-myeloperoxidase (DAKO, Glostrup, Denmark), rabbit polyclonal anti-MnSOD