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Original contributions

Abnormal β-catenin expression in oral cancer with no gene mutation: correlation with expression of cyclin D1 and epidermal growth factor receptor, Ki-67 labeling index, and clinicopathological features

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Summary β-Catenin not only acts as a regulator of E-cadherin–mediated cell-cell adhesion but also plays an important role in Wnt signaling. To assess the prevalence of Wnt signaling, we examined β-catenin mutation and its immunohistochemical protein expression in oral cancers. The results were linked with expression of cyclin D1, one of the target genes of Wnt signaling, expression of epidermal growth factor receptor (EGFR) relevant to β-catenin tyrosine phosphorylation, Ki-67 labeling index, clinicopathological features, and survival. In the analysis based on membranous expression of β-catenin, 75 (68.2%) of 110 cases showed a reduced membranous pattern, and the remaining 35 (31.8%) had a preserved membranous pattern similar to that in oral epithelium. In the analysis of another category of β-catenin expression, a cytoplasmic/nuclear pattern was observed in 21 (19.1%) of the 110 tumors. Most (19/21, 90.5%) of these tumors had a concomitant reduction of membranous expression of β-catenin. The reduced membranous or cytoplasmic/nuclear pattern of β-catenin was significantly associated with an invasive growth pattern, EGFR expression, an increased Ki-67 labeling index, and shorter survival but not with cyclin D1 expression. Mutational analyses of β-catenin were performed for 39 cases, including the 21 tumors with a cytoplasmic/nuclear pattern, but no mutations in the β-catenin gene exon 3 were detected in these samples. Our data indicate that altered expression of β-catenin may play an important

Abbreviations: SCC, squamous cell carcinoma; TCF, T cell factor; LEF, lymphoid enhancer factor; IHC, immunohistochemistry; EGF, epidermal growth factor; EGFR, EGF receptor; EMT, epithelial mesenchymal transition; APC, adenomatous polyposis coli; GSK-3β, glycogen synthase kinase-3β; LI, labeling index.

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role in tumor progression through increased proliferation and invasiveness under EGFR activation. However, mutations of β-catenin do not appear to be responsible for tumor development and abnormal expression of β-catenin in oral cancers.

1. Introduction

β-Catenin, an E-cadherin–mediated cell adhesion molecule, plays an important role in the maintenance of epithelial structure with other members of the E-cadherin/catenin complex [1]. Reduced cell adhesive properties of this complex have been reported to be associated with tumor development and progression in head and neck squamous cell carcinomas (SCCs) [2,3]. In addition, E-cadherin–mediated cell adhesion has been demonstrated to be regulated by the tyrosine phosphorylation of this molecule, which is initially enhanced by epidermal growth factor (EGF) [4]. The EGF receptor (EGFR) directly interacts with β-catenin [5] and the EGFR-induced tyrosine phosphorylation of β-catenin causes dysfunction of the E-cadherin–mediated cell adhesion in cancer, resulting in increased cell motility, invasion, and metastasis [1,6].

β-Catenin also functions as a transcriptional activator of the Wnt signaling pathway in embryonic and tumor development [7,8]. In normal epithelial cells, in the absence of a Wnt signal, free cytoplasmic β-catenin interacts with the adenomatous polyposis coli (APC) tumor-suppressor protein and is phosphorylated by glycogen synthase kinase-3β (GSK-3β) at serine/threonine residues, which are encoded in exon 3 of the β-catenin gene [9,10]. Consequently, β-catenin is rapidly degraded via the ubiquitin-proteasome pathway [11]. On the other hand, activation of Wnt signaling inhibits GSK-3β activity and induces stabilization of cytoplasmic β-catenin, followed by its translocation to the nucleus, forming active transcription complexes that interact with the T-cell factor (TCF)/lymphoid enhancer factor (LEF) [12]. The β-catenin/TCF/LEF complex activates target genes such as cyclin D1 that are involved in the G1-S cell-cycle transition and upregulates cell proliferation [13]. Both mutations in the GSK-3β phosphorylation sites of β-catenin and inactivation of APC similarly cause stabilization of β-catenin, leading to upregulation of TCF/LEF–dependent transcriptional activity [14]. This mutated β-catenin can act as an oncogene and the mutation results in accumulation of the protein in the cytoplasm and/or nucleus of the cancer cell [15,16].

Previously, we observed cytoplasmic/nuclear β-catenin expression in some tumors when we examined expression of the E-cadherin/catenin complex in oral SCCs using immunohistochemistry (IHC) [3]. However, we did not examine mutations in the β-catenin gene with particular emphasis on the alterations of membranous expression of β-catenin [3]. To the best of our knowledge, only one study has examined mutations in β-catenin in head and neck cancer, finding no mutation of its gene [17]. In this study, to examine whether abnormalities in the Wnt/β-catenin signaling pathway occur in oral SCC, we performed mutational analysis of the β-catenin gene and its protein expression with a newly designed category for evaluation in oral SCCs. We also investigated correlations of β-catenin expression with cyclin D1 and EGFR expression, cell proliferative activity assessed by Ki-67, clinicopathological features, and survival.

2. Materials and methods

2.1. Patients and tissue samples

The study group comprised 110 patients with oral SCC who had undergone curative surgery at the department of oral surgery of the Sapporo Medical University School of Medicine (Sapporo, Japan). Case selection was restricted to primary oral SCC for which DNA extraction for molecular analysis was available. The 110 cases included 62 cases (56.4%) examined in our previous study on the expression of the E-cadherin/catenin complex [3]. Patients who died of complications from another cancer or other diseases and those within 1 month after surgery were excluded from the current study. Of the 110 patients, 80 (72.7%) were men and 30 (27.3%) were women The mean age was 59 years (range, 26-85 years). Tumors were located in the tongue in 57 cases (51.8%), in the floor of the mouth in 23 cases (20.9%), in the gingiva in 19 cases (17.3%), in the buccal mucosa in 10 cases (9.1%), and in the lip in 1 case (0.9%). The median follow-up for all patients was 66 months (range, 5-134 months). Tumor size, lymph node status, clinical stage, histological grade (grade of tumor differentiation), and growth pattern (mode of invasion) were available for all patients. The clinical stage, histological grade, and growth pattern were defined according to Union Internationale Contre le Cancer criteria (1997), the World Health Organization tumor classification (2000), and our previous study [3], respectively.

Tumor samples were obtained at the time of either incisional biopsy for initial diagnosis or excisional biopsy under snap-frozen section control. For histopathological and IHC studies, these tumor samples and nontumoral oral mucosal samples were fixed in 10%—buffered—formalin and were then embedded in paraffin.

2.2. Immunohistochemistry

First, 4-μm paraffin sections were dewaxed and dehydrated through ascending grades of ethanol and autoclaved in 0.01 mol/L citrate buffer (pH 6.0) for 15 minutes for heat-based antigen retrieval. After immersion with 3% H_2O_2 solution, a β-catenin monoclonal antibody (C19220, Trans-