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Original contribution

Galectin-1 is a useful marker for detecting neoplastic squamous cells in oral cytology smears☆,☆☆

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Summary Cytologic diagnoses in the oral region are very difficult due to the small amount of cells in smears, which are also exposed to many stimulating factors and often show atypical changes. Galectin-1 (Gal1) is a β-galactoside binding protein that modulates tumor progression. Gal1 is very weakly expressed in normal cells, but is often overexpressed in neoplastic lesions. The aim of the present study was to determine whether it is possible to differentiate reactive changes from neoplastic changes in oral cytology smears based on the expression of Gal1. A total of 155 tissue biopsy specimens and 61 liquid-based cytology specimens were immunostained by an anti-Gal1 antibody, and Gal1 expression levels were subsequently evaluated. These samples consisted of oral squamous cell carcinomas, epithelial dysplasia, and oral mucosal diseases. The positive and negative expressions of Gal1 were examined in 37 specimens collected by scalpel and cytobrush biopsy. The sensitivity, specificity, and positive predictive value of Gal1 were also evaluated in smears. In tissue sections, the positive ratio of Gal1 in neoplastic lesions was high (72.3%). In cytology specimens, the positive ratio of Gal1 was higher in neoplastic lesions (79.0%) than in those negative for intraepithelial lesion or malignancy (22.2%). A correlation was found between immunocytochemical Gal1 expression and immunohistochemical Gal1 expression ($P < .001$). The sensitivity (75.0%), specificity (75.0%), and positive predictive value (91.3%) of Gal1 were also high in smears. In conclusion, Gal1 may be a useful marker for determining whether morphologic changes in cells are reactive or neoplastic.

1. Introduction

Oral squamous cell carcinoma (OSCC) is a frequently encountered malignant lesion and still occupies 10th place in the ranking of cancer-related mortality worldwide. There are an estimated 263 900 new cases and 128 000 deaths annually. OSCC is more common in men than in women, with most cases of OSCC occurring in developing countries [1]. Although OSCC is largely related to lifestyle choices such

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as tobacco and alcohol misuse, genetic and other risk factors have been implicated [1,2].

OSCC is frequently diagnosed at the advanced stage and has a high relapse rate. The most effective strategy to control OSCC is to combine an early diagnosis with timely and appropriate treatments. The definitive diagnosis of OSCC and its precursors (dysplasias) is still mainly based on scalpel biopsy, which may be a contributing factor to the poor prognosis of oral neoplasia because it is invasive and limits sampling to very restricted areas on a small number of sites. Diagnostic oral cytology has been used since the 1960s [3] and is a simple, noninvasive, painless, and inexpensive tool that may also be applied to extensive and multiple lesions. However, the false-positive rate of the oral cytology method has been reported to exceed 30% [4].

This error has been attributed to various factors, including inadequate samplings that are composed of only superficial cells or bloody materials, procedural errors, and the instability of a subjective interpretation of cytologic findings. *Oral cytology* has been defined as the characterization of cells from the surface of the oral mucosa, which is constantly exposed to inflammatory risk factors such as infections (herpes virus and candida), chemicals (tobacco and alcohol), and physical forces (dentures and bite wounds) [2,5].

These factors often induce cytologic and morphologic changes that are reactive or degenerative, similar to neoplastic changes. Therefore, difficulties are associated with separating reactive lesions from neoplastic lesions such as OSCC and epithelial dysplasia (ED).

Previous studies reported that new ancillary techniques such as DNA analyses with flow and image cytometry [6,7], computer-assisted cytology (OralCDx®, CDx Laboratories, Suffern, NY) [8], liquid-based cytology (LBC) [9], and immunocytochemistry [10,11] may improve the sensitivity. In oral cytology, between normal and tumor cells have been morphometrically discriminated from each other using immunocytochemical staining such as that with cytokeratin (CK) 13 and CK17 [10,11], but no studies have examined these markers between oral mucosal diseases (OMDs) and neoplasms. Specific markers that are present in malignant lesions in the oral cavity, but absent in benign lesions and normal mucosa are still limited in number [12]. In order to avoid misdiagnoses, new and useful markers that have the ability to identify reactive or neoplastic changes are needed.

Galectin-1 (Gal1) is a β-galactoside binding protein that mediates cell transformation [13], cell proliferation [14], cell adhesion, angiogenesis [15], immunosuppression [16], and tumor invasiveness [17]. Because of its pleiotropic roles, the up-regulation of Gal1 influences tumor progression [18]. Previous studies showed that Gal1 protein staining was negative in the normal epithelium [15], and that Gal1 protein and messenger RNA levels were increased in various cancer tissues such as those from the colon [19], prostate [20], lung [21], uterine cervix [22], and oral cavity [23].

The aim of the present study was to investigate the expression of Gal1 in OMD and establish whether Gal1 is a useful marker for differentiating reactive changes in oral neoplasms using liquid-based immunocytochemistry.

2. Materials and methods

2.1. Case selection

This study comprised 179 patients who were referred to the Osaka University Dental Hospital. All patients provided informed consent. This study was approved by the institutional review board (IRB No. H20-31). Patients consisted of 82 women and 97 men. The median age of these patients was 66 years (range from 12 to 90 years). The clinical summary for these patients is shown in Table 1. There were 155 tissue specimens in the histologic analysis, which consisted of 49 cases of OSCC, 63 of ED (including 23 low-grade EDs and 40 high-grade EDs), and 43 of OMD such as lichen planus, pemphigus vulgalius, periodontitis, and other inflammatory lesions. Diagnoses were based on the World Health Organization classification [24] and divided into 4 groups: normal ("no neoplasm" including OMD), low-grade ED, high-grade ED, and SCC. Sixty-one oral cytology specimens were included in the cytologic analysis, 37 of which were collected during biopsy and LBC smears. Cytomorphologic features were analyzed by the criteria established according to the Bethesda guidelines (2001): negative for intraepithelial lesions or malignancy (NILM), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and SCC. Their cytologic diagnoses (NILM, LSIL, HSIL, and SCC) were confirmed by histologic diagnoses (OMD, low-grade ED, high-grade ED, and SCC, respectively). Cytology slides were

Table 1 Characteristics of 3 groups of oral lesions analyzed

	Cases of tissue sections	Cases of smears	Cases of tissue sections and smears
Sex			
Male	85	34	22
Female	70	27	15
Age (y)			
Median	66	68	68
Range	26-90	12-87	34-87
Lesion site			
Tongue	60	20	11
Gingival mucosa	60	28	19
Buccal mucosa	19	10	6
Floor of the mouth	5	1	1
Palatal mucosa	7	2	0
Lip	4	0	0
Total	155	61	37