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Emergence of keratin 17 vs. loss of keratin 13: Their reciprocal immunohistochemical profiles in oral carcinoma *in situ*

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SUMMARY

To evaluate differential expressions for keratin (K) subtypes 13 and 17 in oral borderline malignancies, we examined 67 surgical specimens of the oral mucosa for their immunohistochemical profiles. From those specimens, 173 foci of epithelial dysplasia, 152 foci of carcinoma *in situ* (CIS), and 82 foci of squamous cell carcinoma (SCC) were selected according to our diagnostic criteria, along with 20 areas of normal epithelia. In normal epithelia, there was no K17 positivity (0%), whereas definite K13 positivity (100%) was observed. The same tendencies were obtained in mild (undefined) and moderate (true) epithelial dysplasias (K17: 0%; K13: 100%). In contrast, all CIS (100%) had K17 positivities, while K13 positivities were lost in many of them (7%). Similar tendencies were confirmed in invasive SCC (K17: 100%, K13: 4%). Simultaneous immunopositivities for K17 and K13 were found only in SCC (7%) and CIS (4%) foci with distinct keratinization. These foci also showed K10 positivities, though K10 positive areas were not identical to K13 positive areas. The results indicate that expressions of K17 and K13 are reciprocal in oral epithelial lesions and that the K17 emergence is related to malignancies.

Introduction

It has been almost sixty years since Slaughter et al. first introduced the concept of "field cancerization" in 1953.[1] The concept was established based on their histopathological observation that one cancer lesion contained simultaneously multiple foci of carcinoma *in situ* (CIS), which were not continuous with each other and were neighboring foci of epithelial dysplasia or normal epithelial areas. During the intervening years, there have been numerous advances in the field of carcinogenesis, yet it is still challenging to histopathologically distinguish CIS from epithelial dysplasia or from microinvasive carcinoma.[2–5] The boundaries between different grade foci are not always discernible on hematoxylin and eosin (HE)-stained sections, because the malignant changes seem to occur gradually. Although the gradual changes in morphology are often subtle, some molecular events of "cell competition" must occur between cells in the different grades,[6] and hence what is happening in the boundary between such precancerous lesions as CIS and epithelial dysplasia is considered by some researchers to be one of the most interesting subjects in modern cell biology.[7] It is therefore very important to demonstrate functional differences between malignant foci and their neighboring not-yet malignant foci in the oral mucosa before the theory of cell competition is applied to these boundaries.

In spite of many trials using modern technology,[8–12] there is no one-and-only perfect method for making a sharp distinction among these lesions, because actual cancerization steps must also be gradual. Recently, we have proposed a new combined immunohistochemistry for keratin (K) 19 and K13 as markers for cellular differentiation toward basal cells and prickle cells, respectively, and for Ki-67 as a positioning marker for the cell proliferating center in discriminating CIS from epithelial dysplasia after confirming prognoses of individual cases when they were categorized according to the method.[9] Of the immunohistochemical markers, in addition to the three mentioned above, which have been applied to the oral mucosal epithelia, we paid attention to K17 because it was not expressed in normal squamous stratified epithelia[13] but has specifically been immunolocalized in squamous cell carcinomas (SCCs)[14–16] including oral SCC.[17] The purpose of this study was to confirm the immunohistochemical profile of K17 in normal and

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dysplastic epithelia, CIS, and SCC in comparison with that of K13 in those lesions.

Materials and methods

Materials

A total of 177 surgical specimens of the oral mucosa, which had been removed due to SCC or CIS and contained simultaneously areas of epithelial dysplasia and/or normal epithelium in addition to the main SCC/CIS foci, were selected from the surgical pathology files of Niigata University and Sichuan University. Out of the 177 specimens, a total of 427 foci were investigated: 82 were SCC foci, 152 were CIS, 102 were moderate (true) dysplasia, 71 were mild (undefined) dysplasia, and 20 were normal epithelium foci. They were from the tongue (224 foci), gingiva (92), buccal mucosa (58), floor of the mouth (22), oropharynx (15), and lip (16) (Tables 1 and 2). Ten of the maxillary gingiva specimens contained the surgical margins of the palatal mucosa. All the specimens were routinely fixed in 10% formalin and embedded in paraffin. Serial 3-μm sections were cut from paraffin blocks. One set of the sections was stained with hematoxylin and eosin (HE) and was used for reevaluation of histological diagnosis, and the other sets were used for immunohistochemistry.

Immunohistochemistry

Immunohistochemical methods using polymer-immune complexes (EnVision+peroxidase, rabbit/mouse, Dako, Glostrup, Denmark) employed in the present study were previously described elsewhere.[9–12] For control experiments, the primary antibodies were replaced with pre-immune mouse IgG subclasses according to the primary antibody classes (Dako). Mouse monoclonal antibodies against human keratin 17 (K17, clone E3, IgG_{2b}, diluted at 1:20), keratin 13 (K13, clone DE-K13, IgG2a, 1:200), keratin 19 (K19, RCK 108, IgG1, 1:50), and keratin 10 (K10, DE-K10, IgG1, 1:100) were purchased from Dako. A mouse monoclonal antibody against human Ki-67 antigen (clone MIB-1, IgG1, 1:100, Dako) was also used for recognition of cells in proliferating phases.

Table 1
Immunohistochemical profiles for keratins17 and 13 in oral squamous epithelial lesions.

Lesions	Number of areas	Number of foci with immunopositivities for		
		Keratin 17 (%)	Keratin 13 (%)	Keratin 19 (%)
Normal	20	0 (0)	20 (100)	20 (100)
Dysplasia	173	0 (0)	173 (100)	22 (13)
Mild	*71*	*0 (0)*	*71 (100)*	*22 (31)*
Moderate	*102*	*0 (0)*	*102 (100)*	*0 (0)*
Carcinoma *in situ*	152	152 (100)	10 (7)	6 (4)
Basaloid	*36*	*36 (100)*	*3 (8)*	*6 (16)*
Differentiated	*116*	*116 (100)*	*7 (6)*	*0 (0)*
Squamous cell carcinoma	82	82 (100)	3 (4)	0 (0)
Total areas	427			

Histopathological and immunohistochemical evaluation

Using HE and the combined immunohistochemistry for K13, K19, and Ki-67, we reevaluated all the foci for the grading of mild and moderate dysplasia, CIS, and SCC by confirming cellular differentiation toward prickle cells or basal cells, as well as positioning of proliferating zones, respectively,[9] and compared the reevaluated diagnoses with K17 staining results. The definition of CIS covers a wider range of lesions than what was defined by WHO, which seems to be limited to only our basaloid type. According to our diagnostic standards, CIS can be categorized into three subtypes: basaloid, verrucous, and acanthotic; and epithelial dysplasia, on the other hand, can be graded into two classes, mild (undefined) and moderate (true).[9]

Results

Immunohistochemical profiles for five categories of epithelial conditions, (i) normal, (ii) dysplasia, (iii) CIS, and (iv) SCC will be described separately. The immunohistochemical profiles for K19 have not been included because those among the four categories were almost the same as previously described.[9] Table 1 compares the staining results between K17 and K13 among the examined foci.

Normal epithelia

In normal epithelia (Fig. 1A), there were no positivities for K17 (0 of 20 foci, 0%) (Fig. 1B), while K13 was distinctly localized in the epithelial layer except for basal cells (20 of 20 foci, 100%) (Fig. 1C), in which K19 positive (+) cells were aligned (not shown). Ki-67+ cells were sporadically recognized only in the parabasal (second basal) layer (Fig. 1D). K17 was occasionally and faintly positive in the hard palatal mucosa which was contained in the surgical specimens of maxillary gingiva lesions, while K13 was stably positive in the whole mouth. However, these palatal foci were excluded for evaluation, because no borderline malignancies purely arising in the hard palate were found in the present series.

Epithelial dysplasia

Mild (undefined) epithelial dysplasias, defined as epithelia with irregular basal cell alignment but with definite maturation tenden-

Table 2
Intraoral distributions of keratin 13/17-double positive foci among oral squamous epithelial lesions.

Lesions	K13/17+/total focus numbers by intraoral locations						
	Tongue	Gingiva	Buccal	Floor of the mouth	Oropharynx	Lip	Total
Normal	0/5 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/20 (0%)
Epithelial dysplasia	0/95 (0%)	0/45 (0%)	0/22 (0%)	0/5 (0%)	0/3 (0%)	0/3 (0%)	0/173 (0%)
Carcinoma *in situ*	5/76 (7%)	1/30 (3%)	1/22 (5%)	1/11 (9%)	1/7 (10%)	1/6 (14%)	10/152 (7%)
Squamous cell carcinoma	2/48 (4%)	1/14 (7%)	0/11 (0%)	0/3 (0%)	0/2 (0%)	0/4 (0%)	3/82 (4%)
Total	7/224 (3%)	2/92 (2%)	1/58 (2%)	1/22 (5%)	1/15 (6%)	0/16 (0%)	12/427 (3%)