Original Article
Immunohistochemical staining patterns of cytokeratins 13, 14, and 17 in oral epithelial dysplasia including orthokeratotic dysplasia

Aiko Nobusawa,[1,2] Takaaki Sano,[1] Akihide Negishi,[2] Satoshi Yokoo[2] and Tetsunari Oyama[1]

Departments of [1]Diagnostic Pathology and [2]Stomatology and Maxillofacial Surgery, Gunma University Graduate School of Medicine, Maebashi, Japan

Diagnosis of the exact grade of oral epithelial dysplasia is difficult, and interobserver variations in grading are common. The aim of this study was to investigate the expression patterns of cytokeratins (CKs) in dysplastic oral epithelia, to identify useful double immunostaining diagnostic markers. Immunoexpression of CK13, CK14, CK17, and Ki-67 were investigated in 21 normal epithelial specimens and 146 epithelial dysplasia specimens. In epithelial dysplasia specimens, orthokeratotic dysplasia (OKD) was identified using CK10 immunostaining. Most mild dysplasia specimens were CK13+ and CK17–. In moderate dysplasia, CK13 expression tended to be lower and CK17 expression tended to be higher than in mild dysplasia. All carcinoma *in situ* (CIS) specimens were CK17+. In differentiated type CIS specimens, CK13 expression was weakly positive. Most epithelial dysplasia specimens were CK14+. There were no significant differences in the expression patterns of CKs between OKD and non-OKD specimens in any of the grades of dysplasia. These results indicate that CK14 expression can be used to detect early epithelial dysplasia, and that CK13 and CK17 expression are useful for detecting neoplastic changes.

Key words: cytokeratins, double immunostaining, oral epithelial dysplasia, orthokeratotic dysplasia

Cytokeratins (CKs) are cytoskeletal proteins that form the intermediate filaments of epithelial cells. CKs are divided into acidic and basic types,[1] which are coexpressed and arranged as heterodimers. About 30 years ago, Moll *et al.* defined the expression patterns of CKs in normal epithelia, tumors, and cultured human cells.[2] More recently, several studies have reported that these expression patterns correlate with the presence of oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia (OED).[3–12]

Diagnosis of the exact grade of OED is difficult, and interobserver variations in grading are common. OSCC shows specific features of differentiation, while oral carcinoma *in situ* (CIS) shows variations in differentiation.[13] Recently, an oral-type CIS with histological features that differ from the CIS criteria of the World Health Organization (WHO) classification[14] has been reported in retrospective clinicopathological studies.[15] The WHO criteria for CIS include full thickness or almost full thickness architectural abnormalities in the viable cellular layers accompanied by pronounced cytologic atypia.[14] In contrast, the recently reported that oral-type CIS shows well-differentiated cells with squamous cell stratification, with cellular atypia and loss of polarity in the basal layer.[13] The new oral-type CIS is referred to as differentiated type CIS, and may be difficult to diagnose as carcinoma.

Expression of CK13 and CK17 was recently reported to be useful for detecting epithelial neoplasia. CK13 is expressed in normal epithelium and tends to reduce in expression after malignant transformation.[6,8,9] CK17 is not expressed in normal epithelium, but is found in malignant squamous cell lesions of the epithelium.[8,9] CK14 expression was reported in the undifferentiated basal cell layer containing stem cells in normal epithelium.[1,4] Expression of CK13 mRNA is higher in hyperplasia and early dysplasia than in severe dysplasia and OSCC, whereas expressions of CK17 and CK14 are higher in severe dysplasia and OSCC.[16] Although immunohistostaining results are thought to be linked to CK mRNA expression,

few studies have reported on associations between immunohistostaining results and the various grades of epithelial dysplasia.

Recently, a new leukoplakia-type precancerous lesion was reported, termed orthokeratotic dysplasia (OKD).[17] The phenotype of OKD includes orthokeratosis, a granular cell layer, and CK10 positivity. Dysplastic epithelium with orthokeratosis is not particularly rare in oral epithelial specimens. The purpose of this study was to define the immunohistochemical profiles of CK13, CK17, and CK14 in oral epithelial dysplasia including OKD in surgical and biopsy specimens, using double immunostaining.

MATERIALS AND METHODS

Materials

A total of 82 oral mucosal lesion foci collected from 23 uncalcified surgical specimens of squamous cell carcinoma, and 85 oral mucosal biopsy specimens diagnosed as epithelial dysplasia or CIS, were selected from the collection at the Clinical Department of Pathology of Gunma University (Table 1). Twenty-one normal epithelial foci from 23 surgical specimens were also assessed. All specimens were evaluated by three pathologists (A.N., T.S., and T.O.) to gain a consensus diagnosis. Epithelial dysplasia specimens were divided into three groups: mild dysplasia ($n = 43$), moderate dysplasia ($n = 63$), and CIS ($n = 40$). Dysplasia was classified according to the WHO criteria.[14] Mild dysplasia was defined as epithelial dysplasia with architectural disturbance limited to the lower third of the epithelium accompanied by cytologic atypia, moderate dysplasia was defined as architectural disturbance extending into the middle third of the epithelium with upgraded cytologic atypia, and severe dysplasia was defined as architectural disturbance of more than two thirds of the epithelium with cytologic atypia. CIS was defined as full thickness or almost full thickness architectural abnormalities in the viable cellular layers accompanied by pronounced cytologic atypia. The CIS group also included specimens graded as less-differentiated or severe dysplasia according to the WHO classification, because many cases of severe dysplasia were considered to be differentiated type CIS.[13,15] Each specimen

was also categorized as OKD or non-OKD type. Specimens were categorized as OKD type when they showed epithelial dysplasia with orthokeratosis, a granular cell layer, and CK10 positivity, regardless of the grade of dysplasia.[17] For example, moderate dysplasia was classified as OKD type moderate dysplasia or non-OKD type moderate dysplasia.

Antibodies

Four anti-CK antibodies were used to define differentiation in epithelial dysplasia: mouse monoclonal antibodies against human CK13 (clone KS-1A3, 1:40; Leica Microsystems, Newcastle, UK) and human CK10 (clone DE-K10, 1:100; Dako, Glostrup, Denmark), and rabbit monoclonal antibodies against human CK14 (clone EP61, 1:80; Epitomics, Burlingame, CA, USA), and human CK17 (clone EP98, 1:80; Epitomics). A mouse monoclonal antibody against human Ki-67 antigen (clone MIB-1, 1:40; Dako) was used to define the phase of proliferation.

Immunohistochemistry

CK immunohistochemical staining was performed using cocktails of antibodies against CK13 and CK17, and against CK13 and CK17+14. All specimens were fixed in 10% formalin, embedded in paraffin, and cut into 3-μm slices from the paraffin blocks. Sections were deparaffinized with xylene and rehydrated ethanol, and treated with 0.3% hydrogen peroxidase in methanol for 30 min to block endogenous peroxidase activity.

Antigen retrieval was performed by boiling in 0.01 mol/L of citrate buffer at pH 6.0 for 30 min. After three 5-min washes with phosphate-buffered saline (PBS), sections were treated with 5% normal goat serum for 30 min at room temperature to block nonspecific binding sites. All sections were incubated overnight at 4°C with cocktails of antibodies against CK13 and CK17, or against CK13 and CK17+14. Sections were incubated with Simple Stain MAX-PO (mouse) (Nichirei Bioscience, Tokyo, Japan) for 30 min. After three 5-min washes with PBS, sections were incubated with Simple Stain AP (rabbit) (Nichirei Bioscience) for 60 min and rinsed three times with PBS for 5 min each. The peroxidase reaction was performed

Table 1 Distribution of dysplastic lesions and normal epithelia

Locations		Tongue	Ginigiva	Buccal	Floor of the mouth	Hard palate	Soft palate
Type	normal ($n = 21$)	15	2	3	1	0	0
	OKD ($n = 61$)	19	23	9	2	5	3
	non-OKD ($n = 85$)	50	16	16	3	0	0
Total cases ($n = 167$)		84	41	28	6	5	3

non-OKD, mild or moderate dysplasia or carcinoma *in situ* without orthokeratosis; normal, normal epithelia; OKD, orthokeratotic dysplasia with mild or moderate dysplasia or carcinoma *in situ*.

using 0.02% 3-3′-diaminobenzidine tetrahydrochloride for 10 min. After rinsing with distilled water and PBS, the alkali phosphatase reaction was performed using 4% new fuchsin (MP Biomedicals, Santa Ana, CA, USA) for 30 min. Positive staining for CK13 was brown, and positive staining for CK17 and CK17+14 was red. Finally, nuclear counterstaining was performed with Mayer's hematoxylin.

Standard procedures were used for immunohistochemical staining with antibodies against Ki-67 and CK10.[18] Antigen retrieval was performed for CK10 and Ki-67 antibodies by boiling in 0.01 mol/L of citrate buffer at pH 6.0 for 30 min. For Ki-67 antibody, antigen retrieval included additional digestion in 0.1% trypsin. The peroxidase reaction and nuclear counterstaining were performed as described above.

Evaluation of the staining patterns was performed by the same three pathologists for all specimens. Staining for each CK was assessed as positive when more than 10% of the epithelial cells showed intracytoplasmic staining.

Statistical analysis

Fisher's exact test was used to compare differences between the OKD and non-OKD specimens. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Staining patterns were compared between different lesion types (Table 2). In normal epithelia (Fig. 1a), CK13 expression was observed in all specimens (100%, 21/21), with CK13+ cells observed in all layers except the basal layer. No CK17 expression was observed in any normal epithelia (0%, 0/21) (Fig. 1b). CK14+ cells were only observed in the basal layer (Fig. 1c), and Ki-67+ cells were mainly observed in the parabasal layer.

In epithelia with mild dysplasia (Fig. 1d), CK13+ cells were observed in all specimens (100%, 43/43), and CK17+ cells were observed in 12% of specimens (5/43) (Fig. 1e). Expression of CK13 in the lower layers was slightly weaker than in normal epithelia. CK14+ cells were observed in the upper layers and the basal layer. Overexpression of CK14 was

observed in 84% of specimens (36/43) (Fig. 1f). Ki-67+ cells were observed in the basal and parabasal layers.

In epithelia with moderate dysplasia (Fig. 1g), CK13 expression was weakly positive in 51% of specimens (32/63) and CK17 expression was positive in 89% of specimens (56/63) (Fig. 1h). CK17 expression was observed in the middle and upper layers. CK14 expression was observed in all specimens, and CK17+14 expression was observed in all epithelial layers (Fig. 1i). Ki-67+ cells were observed from the basal layer to the lower prickle layer.

All CIS specimens (40/40) (Fig. 2a,d) showed CK17 and CK14 expression. Relatively weak CK13 expression was observed in 11 of the 40 CIS specimens (Fig. 2b,e). Ectopic CK13+ cells were observed only in well-differentiated type CIS specimens (Fig. 2b). Cells expressing CK17+14 were observed in all layers, as for moderate dysplasia. In well-differentiated type CIS, most Ki-67+ cells were located in the basal and parabasal layers (Fig. 2c). In less-differentiated type CIS, Ki-67+ cells were diffusely distributed in the all layers (Fig. 2f).

CK13/CK17 staining patterns were divided into four groups: CK13+/CK17–, CK13–/CK17–, CK13+/CK17+, and CK13–/CK17+ (Fig. 3, left). There was a decrease in the proportion of specimens with CK13+/CK17– and an increase in the proportion of specimens with CK17+ with increasing grades of dysplasia. CK13/CK17+14 staining patterns were analyzed in the same way, and similar results were observed (Fig. 3, right).

Sixty-one of the specimens were categorized as OKD (61/146, 42%) (Fig. 4a, d, Table 2). All CK10+ specimens showed orthokeratosis, and no normal epithelia showed CK10 expression. The most common sites of OKD were the gingiva and the palate (Table 1). There were no significant differences in proportion of each lesion between OKD and non-OKD specimens ($P = 0.14$). CK10+ cells were present in all layers except the basal cell layer (Fig. 4b, e). There were no significant differences in the four CK13/CK17 staining patterns between OKD and non-OKD specimens in any of the grades of dysplasia ($P > 0.05$) (Fig. 5). Among OKD specimens, the proportion of CK13+ specimens tended to decrease and the proportion of CK17+ specimens tended to increase with increasing grades of dysplasia (Fig. 5, left). Forty-seven (77%) of the 61 OKD specimens were CK17+

Table 2 Cytokeratin immunohistochemical staining results

		Total cases				OKD		
	n	CK13 (%)	CK17 (%)	CK17+14 (%)	*n*	CK13 (%)	CK17 (%)	CK17+14 (%)
Normal epithelia	21	21 (100)	0 (0)	0 (0)	–	–	–	–
Mild dysplasia	43	43 (100)	5 (12)	36 (84)	14	14 (100)	2 (14)	13 (93)
Moderate dysplasia	63	32 (51)	56 (89)	63 (100)	32	18 (57)	27 (84)	32 (100)
CIS	40	11 (28)	40 (100)	40 (100)	15	6 (40)	15 (100)	15 (100)

CIS, carcinoma *in situ*; CK, cytokeratin; OKD, orthokeratotic dysplasia with mild or moderate dysplasia or CIS; Total cases, OKD and non-OKD specimens.

HE	CK13/CK17	CK13/17+14
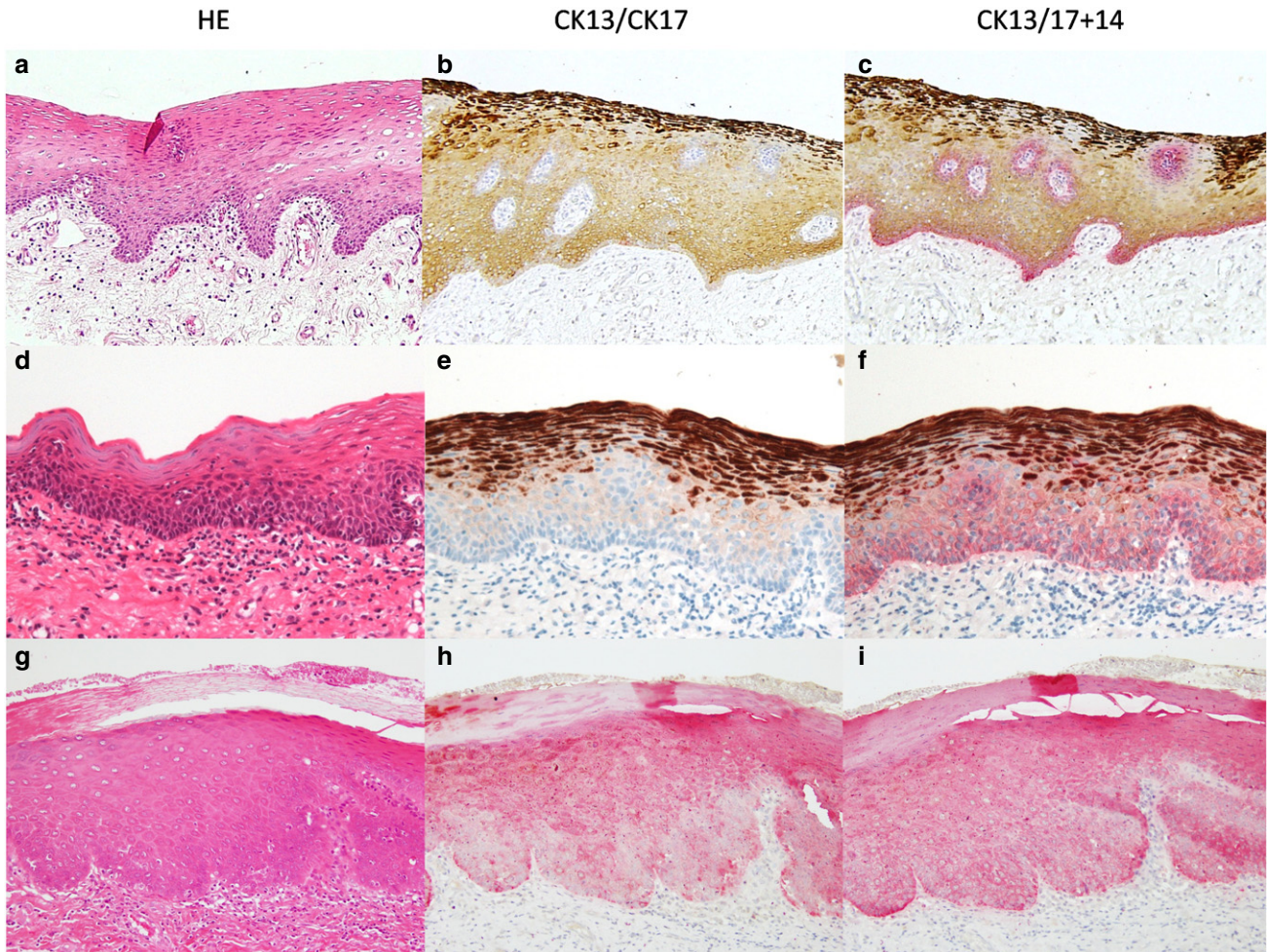
Figure 1 (**a–c**, top row) Staining of normal epithelium, (**d–f**, middle row) mild dysplasia, and (**g–i**, bottom row) moderate dysplasia. (**a,d,g**, left column) Hematoxylin and eosin staining, (**b,e,h**, middle column) double immunostaining for CK13 and CK17, and (**c,f,i**, right column) double immunostaining for CK13 and CK17+14. (**b**) In normal epithelium, CK13 expression was observed in the prickle cell layer to keratinized layer and no CK17 expression was observed, and (**c**) CK14+ cells were observed only in the basal layer. (**e**) A mild dysplasia specimen showing CK13 expression but almost no CK17 expression, with (**f**) CK14+ cells. (**h,i**) A moderate dysplasia specimen showing CK17 and CK14 expression, but no CK13 expression. CK13: brown, CK17 and CK17+14: red.

(Fig. 4c). Three of the OKD specimens were CK17–/CK13–, and all these specimens showed moderate atypia (Fig. 4d–f).

DISCUSSION

CK13 paired with CK4 is an important component of mucosal stratified squamous epithelium.[1] In normal epithelia, CK13 is expressed from the prickle cell layer to the keratinized layer, and CK13+ cells are present during normal differentiation and keratinization.[1,8] In dysplastic epithelia that become cancerous, cytoskeletal changes alter the cellular shapes. Down-regulation of CK13 expression may therefore reflect the presence of atypical cells. In the present study, CK13 expression was reduced in moderate dysplasia and CIS, indicating that abnormal differentiation occurs in dysplastic epithelia.

However, CK13 expression was observed in some well-differentiated CIS specimens.

CK17 is a type I cytokeratin that plays important roles in fetal epidermal development, skin wound healing, and dermal reactions.[19,20] CK17 expression has been demonstrated in various types of normal epithelia, such as the basal cells of transitional and pseudostratified epithelia of the respiratory and urinary tracts.[1] CK17 is also expressed in squamous cell carcinoma of the oral cavity, esophagus,[21] lung,[22] and uterine cervix.[23–25] A previous study reported that CK17 regulated the size and growth of keratinocytes by binding to the adaptor protein 14-3-3 sigma and stimulating the mTOR pathway.[20] This pathway plays a central role in the control of protein synthesis and cell growth, and stimulation causes rapid cell growth during tissue repair of injuries. Sakamoto et al. found that CK17 expression increased cell mobility and
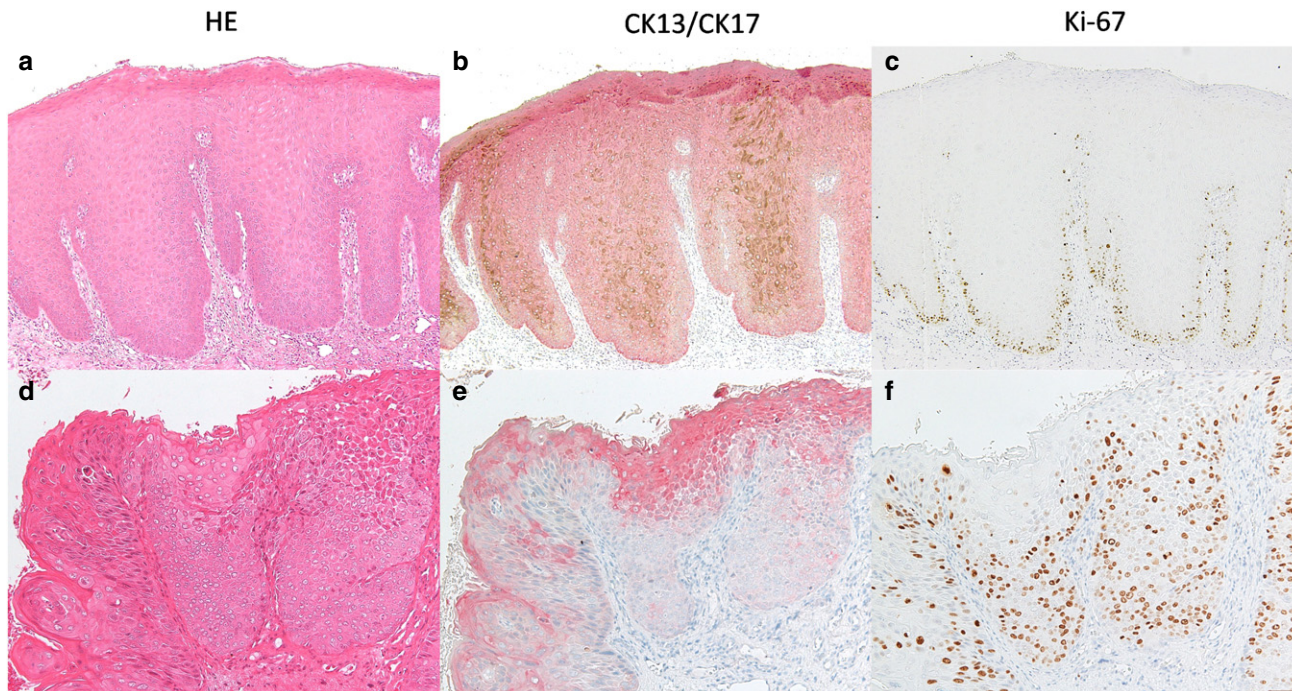
Figure 2 (**a–c**, top row) Staining for well-differentiated type carcinoma *in situ* (CIS) and (**d–f**, bottom row) less-differentiated type CIS. (**a,d**, left column) Hematoxylin and eosin staining, (**b,e**, middle column) double immunostaining for CK13+17, and (**c,f**, right column) staining for Ki-67. (**b,e**) CIS specimens were CK17+. In well-differentiated type CIS, (**b**) CK13+ keratinizing cells were only observed in the prickle layer, and (**c**) Ki-67+ cells were only observed in the basal and parabasal layers. In less-differentiated type CIS, (**e**) no CK13+ cells were observed, and (**f**) Ki-67+ cells were diffusely distributed from the basal layer to the upper prickle layer. (**b,e**) CK13: brown, CK17: red.
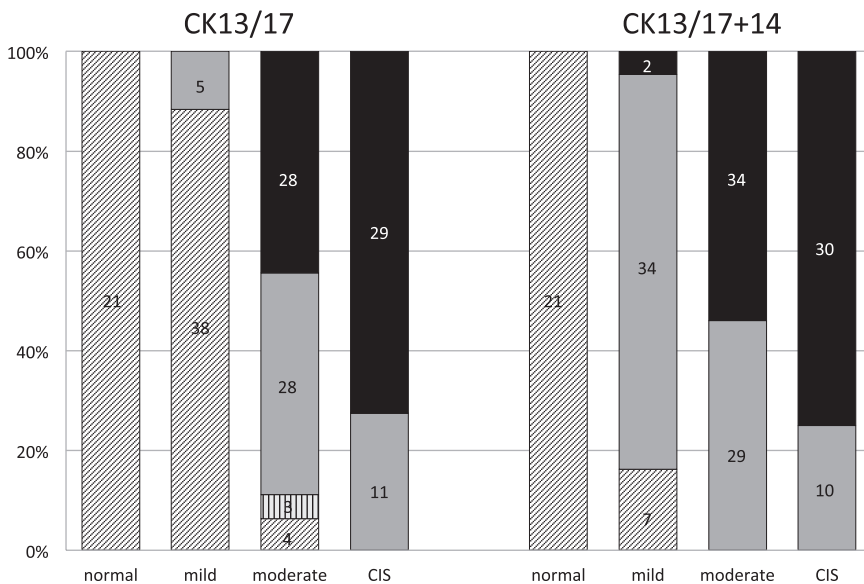

Figure 3 Expression patterns of (left) CK13 and CK17, and of (right) CK13 and CK17+14, in mild and moderate dysplasia and carcinoma *in situ* (CIS). CK13 expression tended to decrease and CK17 expression tended to increase with increasing grades of dysplasia (left). Staining patterns for CK13/CK17+14 (right) were similar to those of CK13/CK17. ■, –/+; ▨, +/+; ▥, –/–; ▧, +/–.

migration, indicating that it might lead to architectural alterations in dysplastic epithelia and carcinoma.[9]

In the present study, strong expression of CK17 was observed in moderate dysplasia and CIS. This suggests that CK17 expression may be a highly specific marker for neoplastic change. Ki-67 expression is also useful for detecting neoplastic lesions because it marks proliferating cells. However, there are relatively few Ki-67+ cells in well-differentiated type CIS, where they are mainly observed in the basal and parabasal layers. CK17 expression is therefore more useful for detecting neoplastic lesions, especially CIS and carcinoma, than Ki-67 expression.
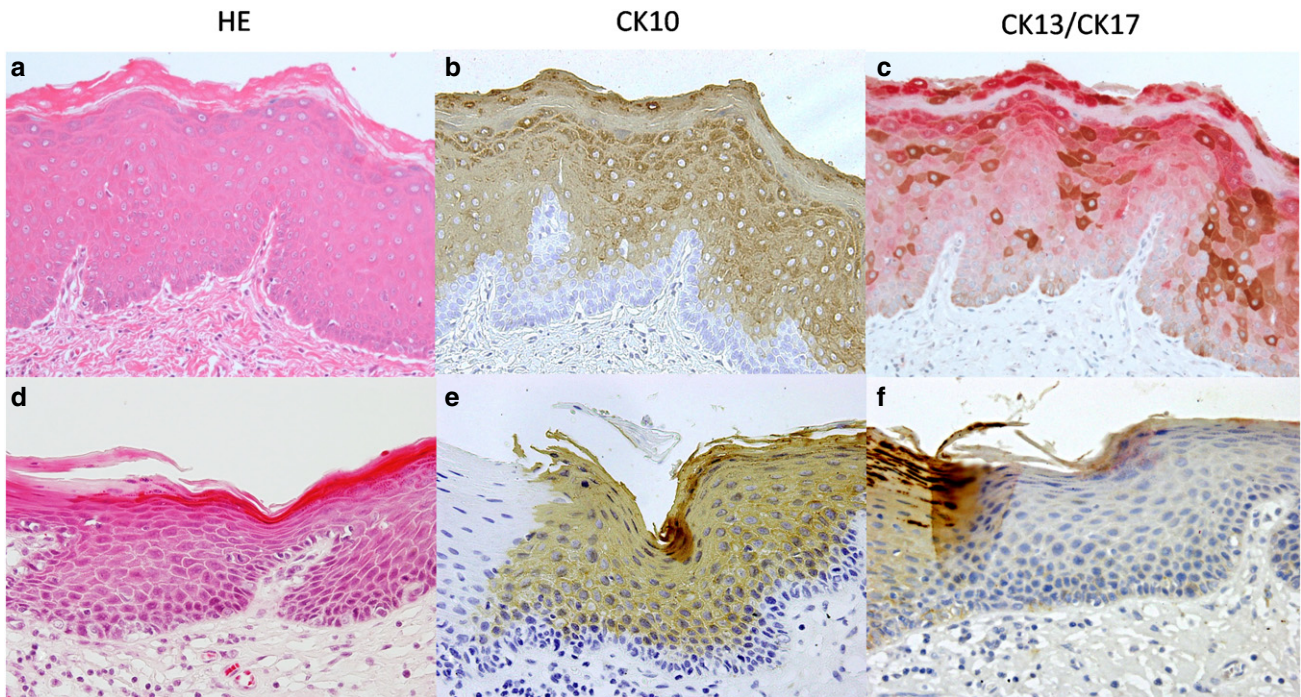
Figure 4 (**a,d**) Hematoxylin and eosin (HE) and (**b,c,e,f**) immunohistochemical staining of two orthokeratotic dysplasia specimens. (**a,d**) HE staining showed orthokeratosis and a granular cell layer with dysplastic changes. (**b**) The specimen shown in the top row showed CK10 expression in all layers except the basal layer, (**c**) CK17 expression, and weak CK13 expression. The specimen shown in the bottom row showed (**e**) CK10 expression, but no (**f**) CK13 or CK17 expression. CK13: brown, CK17: red (**c,f**).
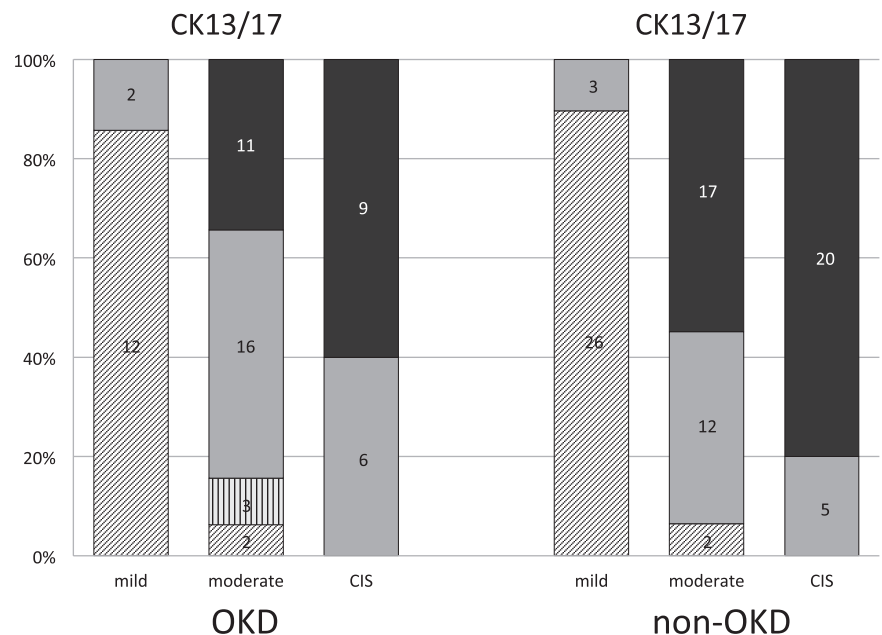
Figure 5 Expression patterns of CK13 and CK17 in orthokeratotic dysplasia (OKD) (left) and non-OKD (right) specimens. The expression patterns tended to be similar in OKD and non-OKD specimens, with no significant differences between the two groups in any of the types of dysplasia. ■, –/+; ▧, +/+; ▥, –/–; ▨, +/–.

CK14 is expressed in the basal layer of normal epithelia. Together with CK5, it forms the main component of hemidesmosomes, and mutations in these CKs are responsible for the skin disease epidermolysis bullosa simplex. CKs are therefore important for the maintenance of basal layer integrity. In the epidermis, CK5 and CK14 are expressed in undifferentiated basal keratinocytes.[26] CK14 is also expressed in the basal layer of normal oral epithelia.

In the present study, CK14 expression was observed in the upper layers of dysplastic epithelia, reflecting abnormal cellular differentiation. This is consistent with the results of a previous study that found increased CK14 mRNA expression in dysplasia and OSCC,[16] resulting in speculation that control of CK14 expression may be disrupted during dysplastic changes. Another study reported positive CK14 immunostaining in dysplastic epithelia including mild dysplasia.[4] Similarly, overexpression of CK14 was observed even in early dysplasia in our study. Although CK14 expression is considered to be useful for the early detection of epithelial dysplasia because of its high sensitivity to dysplastic change, it does not necessarily indicate the grade of dysplasia.

In this study, double immunostaining allowed us to clearly observe differences in the expression patterns of CK13 and CK17 in lesions of different grades. Our results may be useful for the grading of intraepithelial lesions of the oral mucosa. Dysplastic lesions with CK13–/CK17– were infrequent (3/167), and all three of these lesions had OKD. In contrast, 47 of the 61 OKD lesions were CK17+. CK10 is a major keratin involved in keratinocyte differentiation and keratinization, and is considered to be a marker of keratinization.[1] CK10 is expressed in the parabasal layer of the epidermis, and imparts mechanical integrity to the epidermis, but is not expressed in normal oral mucosa.[1,2] CK10 expression was previously reported in keratin pearls in squamous cell carcinoma of the head and neck,[27] suggesting that CK10 expression in oral mucosa indicates abnormal keratinization. Kobayashi *et al.* reported that CK10+ in OKD indicated abnormal keratinization, and suggested that OKD is not a true malignancy as it does not show expression of CK13 or CK17.[17] We used the same antibody against CK10 in our study as the one used by Kobayashi *et al.*, and this antibody contains no clones that react with CK13 or CK17 antigenic sites. The most common site of OKD in our study was the gingiva, which is consistent with the findings reported by Kobayashi *et al.* However, we observed only three OKD specimens that were CK13–/CK17–. Among OKD specimens, the proportion of specimens that were CK17– was highest in mild dysplasia specimens, and the proportion of specimens that were CK17+ increased with increasing grades of dysplasia, as in non-OKD specimens. Moreover, there were no significant differences between the OKD and non-OKD specimens in the ratios of CK13 and CK17 expression in any of the grades of dysplasia. This indicates that OKD includes both low-grade dysplasia, and high-grade dysplasia suggestive of neoplastic lesions.

In conclusion, CK14 immunohistochemical staining can be useful for the detection of early epithelial dysplasia. CK13–/CK17+ staining suggests neoplasia, but not all CK13–/CK17+ lesions are neoplastic. Some OKD lesions are CK17+, indicating that OKD includes neoplastic lesions.

REFERENCES

1 Moll R, Divo M, Langbein L. The human keratins: Biology and pathology. *Histochem Cell Biol* 2008; **129**: 705–33.
2 Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982; **31**: 11–24.
3 Marley JJ, Robinson PA, Hume WJ. Expression of human cytokeratin 14 in normal, premalignant and malignant oral tissue following isolation by plaque differential hybridisation. *Eur J Cancer B Oral Oncol* 1994; **30**: 305–11.
4 Farrar M, Sandison A, Peston D, Gailani M. Immunocytochemical analysis of AE1/AE3, CK 14, Ki-67 and p53 expression in benign, premalignant and malignant oral tissue to establish putative markers for progression of oral carcinoma. *Br J Biomed Sci* 2004; **61**: 117–24.
5 Su L, Morgan PR, Lane EB. Keratin 14 and 19 expression in normal, dysplastic and malignant oral epithelia. A study using in situ hybridization and immunohistochemistry. *J Oral Pathol Med* 1996; **25**: 293–301.
6 Ohta K, Ogawa I, Ono S *et al.* Histopathological evaluation including cytokeratin 13 and Ki-67 in the border between Lugol-stained and -unstained areas. *Oncol Rep* 2010; **24**: 9–14.
7 Kobayashi T, Maruyama S, Cheng J *et al.* Histopathological varieties of oral carcinoma in situ: Diagnosis aided by immuno-histochemistry dealing with the second basal cell layer as the proliferating center of oral mucosal epithelia. *Pathol Int* 2010; **60**: 156–66.
8 Mikami T, Cheng J, Maruyama S *et al.* Emergence of keratin 17 vs. loss of keratin 13: Their reciprocal immunohistochemical profiles in oral carcinoma in situ. *Oral Oncol* 2011; **47**: 497–503.
9 Sakamoto K, Aragaki T, Morita K *et al.* Down-regulation of keratin 4 and keratin 13 expression in oral squamous cell carcinoma and epithelial dysplasia: A clue for histopathogenesis. *Histopathology* 2011; **58**: 531–42.
10 Kitamura R, Toyoshima T, Tanaka H *et al.* Association of cytokeratin 17 expression with differentiation in oral squamous cell carcinoma. *J Cancer Res Clin Oncol* 2012; **138**: 1299–310.
11 Wei KJ, Zhang L, Yang X *et al.* Overexpression of cytokeratin 17 protein in oral squamous cell carcinoma in vitro and in vivo. *Oral Dis* 2009; **15**: 111–17.
12 Sakamoto K, Fujii T, Kawachi H *et al.* Reduction of NOTCH1 expression pertains to maturation abnormalities of keratinocytes in squamous neoplasms. *Lab Invest* 2012; **92**: 688–702.
13 Izumo T. Oral premalignant lesions: From the pathological viewpoint. *Int J Clin Oncol* 2011; **16**: 15–26.
14 Gale N, Westra W, Pilch BZ *et al.* Epithelial precursor lesions. In: Barnes L, Everson JW, Reichart P, Sindransky D, eds. *World Health Organization Classification of Tumours: Pathology and Genetics of Head and Neck Tumours.* Lyon: IARC Press, 2005; 177–9.
15 Japan Society for Oral Tumors. *General Rules for Clinical and Pathological Studies on Oral Cancer*, 1st edn. Tokyo: Kanehara, 2010 (in Japanese).
16 Ohkura S, Kondoh N, Hada A *et al.* Differential expression of the keratin-4, -13, -14, -17 and transglutaminase 3 genes during the development of oral squamous cell carcinoma from leukoplakia. *Oral Oncol* 2005; **41**: 607–13.

17 Kobayashi T, Maruyama S, Abé T *et al*. Keratin 10-positive orthokeratotic dysplasia: A new leucoplakia-type precancerous entity of the oral mucosa. *Histopathology* 2012; **61**: 910–20.

18 Sano A, Sakurai S, Kato H *et al*. Clinicopathological and immunohistochemical characteristics of esophageal carcinosarcoma. *Anticancer Res* 2009; **29**: 3375–80.

19 Ide M, Kato T, Ogata K, Mochiki E, Kuwano H, Oyama T. Keratin 17 expression correlates with tumor progression and poor prognosis in gastric adenocarcinoma. *Ann Surg Oncol* 2012; **19**: 3506–14.

20 Kim S, Wong P, Coulombe PAA. Keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature* 2006; **441**: 362–5.

21 Takahashi H, Shikata N, Senzaki H, Shintaku M, Tsubura A. Immunohistochemical staining patterns of keratins in normal oesophageal epithelium and carcinoma of the oesophagus. *Histopathology* 1995; **26**: 45–50.

22 Wetzels RH, Schaafsma HE, Leigh IM *et al*. Laminin and type VII collagen distribution in different types of human lung carcinoma: Correlation with expression of keratins 14, 16, 17 and 18. *Histopathology* 1992; **20**: 295–303.

23 Carrilho C, Alberto M, Buane L, David L. Keratins 8, 10, 13, and 17 are useful markers in the diagnosis of human cervix carcinomas. *Hum Pathol* 2004; **35**: 546–51.

24 Ikeda K, Tate G, Suzuki T, Mitsuya T. Coordinate expression of cytokeratin 8 and cytokeratin 17 immunohistochemical staining in cervical intraepithelial neoplasia and cervical squamous cell carcinoma: An immunohistochemical analysis and review of the literature. *Gynecol Oncol* 2008; **108**: 598–602.

25 Smedts F, Ramaekers F, Troyanovsky S *et al*. Keratin expression in cervical cancer. *Am J Pathol* 1992; **141**: 497–511.

26 Uitto J, Richard G, McGrath JA. Diseases of epidermal keratins and their linker proteins. *Exp Cell Res* 2007; **313**: 1995–2009.

27 Chovanec M, Plzák J, Betka J, Brabec J, Kodet R, Smetana K Jr. Comparative analysis of alpha2,3/2,6-linked N-acetylneuraminic acid and cytokeratin expression in head and neck squamous cell carcinoma. *Oncol Rep* 2004; **12**: 297–301.