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Expression of BUBR1 in human oral potentially malignant disorders and squamous cell carcinoma

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Objective. BUBR1 is one of the key components of the spindle assembly checkpoint (SAC) machinery and is activated in response to kinetochore tension. Defects in the SAC contribute to an increased rate of aneuploidization during tumorigenesis. The aim of the present study was to examine the immunohistochemical expression of BUBR1 protein for human oral squamous cell carcinogenesis.

Study design. A total of 120 samples of squamous cell carcinoma (SCC, n = 43) and 5 types of potentially malignant disorders (PMDs: oral epithelial dysplasia, n = 11; hyperkeratosis/epithelial hyperplasia, n = 20; lichen planus, n = 16; submucous fibrosis, n = 19; and verrucous hyperplasia, n = 11) of human oral mucosa (1991-2001) from our institution were retrieved and immunohistochemical staining were performed. Normal oral mucosa (n = 9) and fibrous hyperplasia (n = 9) from patients without the aforementioned oral habits were also included in the study.

Results. BUBR1 staining was detected at the basal and suprabasal layers in 75 (97.4%) of 77 samples of PMD and 43 (100%) of 43 samples of SCC of oral mucosa but was absent in all samples of normal oral mucosa (n = 9) and fibrous hyperplasia (n = 9). BUBR1 expression of various types of PMD and SCC of oral mucosa was significantly overexpressed as compared respectively with normal mucosa ($P < .001$) and fibrous hyperplasia ($P < .001$). Moreover, the expression of oral SCC was significantly higher as compared respectively with the 5 types of oral PMD; on the other hand, BUBR1 expression of verrucous hyperplasia was significantly higher than that of the other 4 types of PMD of oral mucosa ($P < .001$).

Conclusion. Our results may interpret that BUBR1 protein is suggested to be one of the contributing factors involved in the pathogenesis of oral SCC. These also hypothesize that BUBR1 protein is a putative biomarker for human oral squamous cell carcinogenesis. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:257-267)**

Squamous cell carcinoma (SCC) of the oral cavity is a major health problem, as indicated by its high incidence in many parts of the world. Oral SCC has been ranked the 12th most common cancer in the world[1] and the 8th most common in males.[2] Potentially malignant disorders (PMDs)[3] of human oral mucosa include lichen planus,[4] submucous fibrosis,[5] leukoplakia,[6] and verrucous hyperplasia.[7] Dysplastic lesions of the oral cavity

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Table I. Demographic characteristics of the collected samples

	Oral potentially malignant disorders					*Oral squamous cell carcinomas*		
						Differentiation		
	Submucous fibrosis	*Hyperkeratosis/epithelial hyperplasia*	*Lichen planus*	*Epithelial dysplasia*	*Verrucous hyperplasia*	*Well*	*Moderate*	*Poor*
Sex								
Male	19	18	15	10	11	20	16	5
Female	0	2	1	1	0	1	0	1
Anatomical sites								
Lip	0	11	2	7	4	0	1	1
Buccal mucosa	18	9	14	3	4	10	8	0
Alveolus/gingiva	0	0	0	0	0	2	0	0
Tongue	1	0	0	1	1	3	2	2
Others	0	0	0	0	2	6	5	3
Age, y, mean ± SD (range)*	39.6 ± 13.6 (22-68)	49.3 ± 13.4 (18-74)	46.7 ± 16.5 (21-78)	54.9 ± 15.7 (24-75)	46.1 ± 11.1 (29-66)	50.2 ± 12.7 (32-79)	49.1 ± 9.6 (34-70)	57.3 ± 12.0 (42-76)

*Age difference was statistically significant when comparing submucous fibrosis with hyperkeratosis/epithelial hyperplasia and oral epithelial dysplasia with oral squamous cell carcinoma (1-way analysis of variance, $P < .03$).

range from mild to moderate and severe dysplasia,[8] and the presence and degree of dysplasia are often used to predict malignant transformation.[9]

Development of SCC is characterized by complex chromosomal instability with structural and numerical variations. The kinetochore is defective for movement and causes chromosomes to lag at both the metaphase and anaphase of the cell cycle.[10] The presence of a single unattached kinetochore activates the spindle assembly checkpoint (SAC), providing additional time for kinetochores to be captured by the mitotic spindle protein, Mad2, and for tension to be sought out by BUBR1.[11] It has been suggested that ongoing genetic instability in terms of chromosome number and structure is a consistent feature of primary head and neck tumors and oral cancer cell lines.[12-14]

The *hBUB1B* gene, which encodes BUBR1, is located on human chromosome 15q14-21, which is a region with a high incidence of loss of heterozygosity (LOH) associated with several tumors, including carcinomas of the colorectum, urinary bladder, breast, lung, and head and neck.[15] The *hBUB1B* gene contains a C-terminal serine-threonine protein kinase domain that is highly homologous to BUB1 protein.[16] BUBR1 protein is a key component of the SAC machinery, which restrains cells from entering the anaphase until all chromosomes are properly attached to bipolar spindles and is activated in response to kinetochore tension.[11] BUBR1 has been shown to be concentrated in the outer kinetochore plate throughout mitosis.[17,18] Li et al.[19] revealed that *hBUB1B* is expressed in various human tissues with a high mitotic index, such as human fetal tissues, but not in differentiated tissues. Thus, *hBUB1B* gene expression is usually undetectable in normal tissues. During the course of natural aging, a marked decline in BUBR1 protein expression has been found in mouse tissues, suggesting a possible role of BUBR1 in regulating natural aging[18-21] that has been defined as an emergency defense system for cells on the way to becoming cancerous.[22]

Overexpression of BUBR1 protein has been observed in many types of cancers,[18,23-28] but not for oral SCC, to our knowledge. Therefore, the relationship between BUBR1 protein expression and human oral squamous cell carcinogenesis remains to be elucidated. In the current study, immunohistochemical expression of BUBR1 protein in various PMDs and SCC for human oral mucosa was evaluated.

MATERIALS AND METHODS

Tissue sample collection

Samples from 120 patients (male: 114; female: 6) with PMDs (n = 77) and SCC (n = 43) of human oral mucosa (1991-2001) were retrieved with the approval of our Institution (Table I). All of the patients from whom the samples originated had the oral habits of betel quid chewing, alcohol drinking, and cigarette smoking with the exception of those patients of lichen planus. The 77 cases of PMD comprised lichen planus (n = 16), oral submucous fibrosis (n = 19), hyperkeratosis/epithelial hyperplasia (n = 20), verrucous hyperplasia (n = 11), and oral epithelial dysplasia (n = 11). The histopathological characteristics of epithelial dysplasia include (1) basal layer hyperplasia, (2) nuclear enlargement and hyperchromatism, (3) loss of intercellular adhesion and normal polarization, (4) abnormal mitoses above the basal cell layer, (5) individual cell keratinization within the spinous layer, (6) cellular pleomorphism, (7) drop-shaped epithelial ridges, (8) irregular stratification, and (9) altered nuclear-cytoplasmic ratio.[29] For these histological changes, the presence of basal cell hyperplasia, nuclear enlargement and hyper-

chromatism, and drop-shaped rete-ridges are regarded as the minimal criteria for the histological diagnosis of epithelial dysplasia.[30] The degrees of dysplasia were graded with reference to the following criteria[31]: (1) mild epithelial dysplasia: dysplastic alterations limited to the lower third of the buccal epithelium; (2) moderate epithelial dysplasia: dysplastic changes noted for up to two thirds of the thickness of the oral epithelium; and (3) severe dysplasia: dysplastic cells observed within more than two thirds but less than the whole thickness of the oral epithelium. Hyperplastic/hyperkeratotic epithelial lesions were comprised for clinical reasons because leukoplakia, the best known oral premalignant lesion, is most commonly associated with clinical diagnosis of epithelial hyperplasia/hyperkeratosis.[6] The histological criteria for verrucous hyperplasia were[7] (1) epithelial hyperplasia with parakeratosis/hyperkeratosis and verrucous surface, and (2) no invasion of the hyperplastic epithelium into the lamina propria as compared with adjacent normal oral epithelium. Oral submucous fibrosis was histologically characterized by epithelial atrophy and progressive deposition of collagen in the lamina propria and submucosa of the oral mucosa.[5] Histological characteristics of lichen planus included[4] (1) stratified squamous epithelium with areas of acanthosis and hyperkeratosis, (2) the presence of a dense lymphocytic inflammatory infiltrate along the epithelium/connective-tissue interface, (3) hydropic degeneration of the basal layer, and (4) some necrotic keratinocytes. There were no signs of dysplasia in lichen planus lesions. Histological differentiation of oral SCC was categorized as well (n = 21), moderate (n = 16), or poor (n = 6). The histological diagnoses of all samples were confirmed by 2 board-certified oral pathologists from hematoxylin and eosin (H&E)-stained sections. Samples of normal oral mucosa (n = 9) and fibrous hyperplasia (n = 9) from patients without the aforementioned oral habits were also included in the study.

Semiquantitative immunohistochemistry for BUBR1 protein

For the detection of BUBR1 protein, a labeled streptavidin biotin (LSAB) technique was used.[32] A 4-μ-thick section of each paraffin-embedded case was prepared. All tissue sections were deparaffinized and dehydrated with a descending series of alcohol. Antigen retrieval was performed in a citrate buffer (pH 6.0) using an electric pressure cooker (Tissue-Tek DRS; Sakura, Torrance, CA, USA) for 10 minutes at 120°C, with cooling for 10 minutes before immunostaining. Tissue sections were then incubated with 3% hydrogen peroxide for 5 minutes to eliminate endogenous peroxidase, followed by primary BUBR1 antibody of 1:150 dilution (Cat. No. 612503, BD Biosciences Pharmingen, Franklin Lakes, NJ, USA) for 30 minutes, horseradish peroxidase–labeled polymer for 30 minutes, and diaminobenzidine as chromogen for 5 minutes, and were then counterstained with Dako automation hematoxylin for 15 minutes (Dako, Carpinteria, CA, USA). Between incubations, all tissue sections were washed with Tris-buffered saline (TBS) buffer. A section of lymph node was used as the positive control and ensured the reproducibility of the staining process. Sections incubated without the primary antibody served as negative controls.

The BUBR1-stained sections were compared with the corresponding H&E-stained sections to establish a topographic relationship between BUBR1-stained areas and histopathological diagnoses. To enumerate the BUBR1-stained cells, 300 cells were examined in at least 5 areas (slides were divided into 9 equal areas under the microscope) at ×400 magnification and a mean percentage of positive-stained cells was determined; each sample was then assigned to 1 of the following 6 staining scores: 0 (less than 10%), 1 (10%-25%), 2 (26%-50%), 3 (51%-75%), 4 (76%-90%), or 5 (91%-100%). Furthermore, BUBR1-positive staining in PMDs was categorized as one of the following levels: not detectable, basal cell layer, lower suprabasal (up to 3-4 layers), and upper suprabasal (up to 5-6 layers) layer staining.

Statistical analysis was performed using SPSS version 14 software (SPSS Inc, Chicago, IL). BUBR1-positive samples of the different age groups were compared by 1-way analysis of variance (ANOVA) followed by Fisher's least significant difference comparison. The numbers of positive-stained cells in the different groups were compared using Student t test. The results were considered significant when the P value was less than .05.

RESULTS

Demography

The demographic data of the patients from whom all samples were taken are shown in Table I. Most of our cases were male (male-to-female ratio = 19:1); the youngest case was an 18-year-old patient with hyperkeratosis/epithelial hyperplasia, whereas the eldest patient was a 79-year-old with well-differentiated oral SCC. The age differences when comparing submucous fibrosis with hyperkeratosis/epithelial hyperplasia, oral epithelial dysplasia, and oral SCC were statistically significant (1-way ANOVA analysis, $P < .03$). The most common site of occurrence of both oral PMD and SCC was the buccal mucosa, followed by the lip for oral PMD and the tongue for oral SCC.

Table II. Scores of immunohistochemical expression of BUBR1 protein of the collected samples

	Normal mucosa/fibrous hyperplasia	*Oral potentially malignant disorders*					*Oral squamous cell carcinoma*		
		Submucous fibrosis	*Hyperkeratosis/epithelial hyperplasia*	*Lichen planus*	*Epithelial dysplasia*	*Verrucous hyperplasia*	*Differentiation*		
							Well	*Moderate*	*Poor*
No.	9	19	20	16	11	11	21	16	6
0%	9	0	0	0	0	0	0	0	0
<10%	0	1	1	0	0	0	0	0	0
10~25%	0	2	4	6	1	0	0	0	0
26~50%	0	14	9	2	6	2	0	0	0
51~75%	0	2	6	6	4	9	0	0	0
76~90%	0	0	0	2	0	0	0	0	0
91~100%	0	0	0	0	0	0	21	16	6

Table III. Immunohistochemical expression of BUBR1 protein of potentially malignant disorders of human oral mucosa with respect to the epithelial layers

	Potentially malignant disorders (N = 77)				
	Submucous fibrosis	*Hyperkeratosis/epithelial hyperplasia*	*Lichen planus*	*Epithelial dysplasia*	*Verrucous hyperplasia*
No.	19	20	16	11	11
Not detectable	1	1			
	5.26%	5.0%			
Basal layer	6	5	8	1	1
	31.58%	25.0%	50.0%	9.09%	9.09%
Lower suprabasal	12	14	8	9	9
	63.16%	70.0%	50.0%	81.82%	81.82%
Upper suprabasal				1	1
				9.09%	9.09%

Semiquantitative immunochemistry for BUBR1 protein

The immunohistochemical expression scores based on the percentage of positive-stained cells (not the staining intensity) for all samples are shown in Table II, and the numbers of samples showing positive staining at the basal and suprabasal layers for the 5 types of oral PMD are shown in Table III.

BUBR1 staining was not detected in any samples of normal oral mucosa (n = 9) and fibrous hyperplasia (n = 9) (Tables II and III; Fig. 1, *b*); in contrast, cytoplasmic BUBR1 staining with occasional nuclear staining was detected at the basal and suprabasal layers (Tables II and III; Figs. 1, *d, f*; 2, *b, d, f*, and 3, *b, d, f*) in nearly all of the samples of PMD of oral mucosa (75/77, 97.4%; Table II). A total of 54 samples of oral PMD revealed suprabasal layer BUBR1 staining and 21 samples exhibited basal layer staining (Table III). Suprabasal layer staining for BUBR1 protein was demonstrated in most cases of oral epithelial dysplasia and verrucous hyperplasia (~90%, each), followed by hyperkeratosis/epithelial hyperplasia (70%), submucous fibrosis (~63%), and lichen planus (50%) (Table III). All samples of oral SCC demonstrated positive BUBR1 staining (43/43, 100%; Table II) with chiefly cytoplasmic but occasional nuclear staining (Fig. 4, *b, d, f*). Positive staining was also observed for the abnormal mitotic cells (Fig. 4, *b, d, f*). No difference in BUBR1 staining was noted for the well-, moderately, and poorly differentiated oral SCC (Table II).

Statistically, BUBR1 expression for each of the 5 types of PMD and SCC of oral mucosa showed significant overexpression as compared with that of normal mucosa ($P < .001$, Table IV). BUBR1 expression of oral SCC was also significantly higher as compared respectively with each of the 5 types of oral PMD ($P < .001$, Table IV). Notably, significant correlations of BUBR1 expression were noted only for verrucous hyperplasia when compared with the other 4 types of PMD ($P < .001$, Table IV).

DISCUSSION

An increase in chromosomal segregation errors during the mitotic process would lead to chromosomal instability, which is frequently related to human malignancies.[33,34] Defects in BUBR1 contribute to chromosomal instability[35]; furthermore, BUBR1 has been found to have a pivotal function in mitotic defects, giving a checkpoint response for cells with centrosome amplification.[36] In the current study, overexpression of BUBR1 protein was observed not only for PMDs but

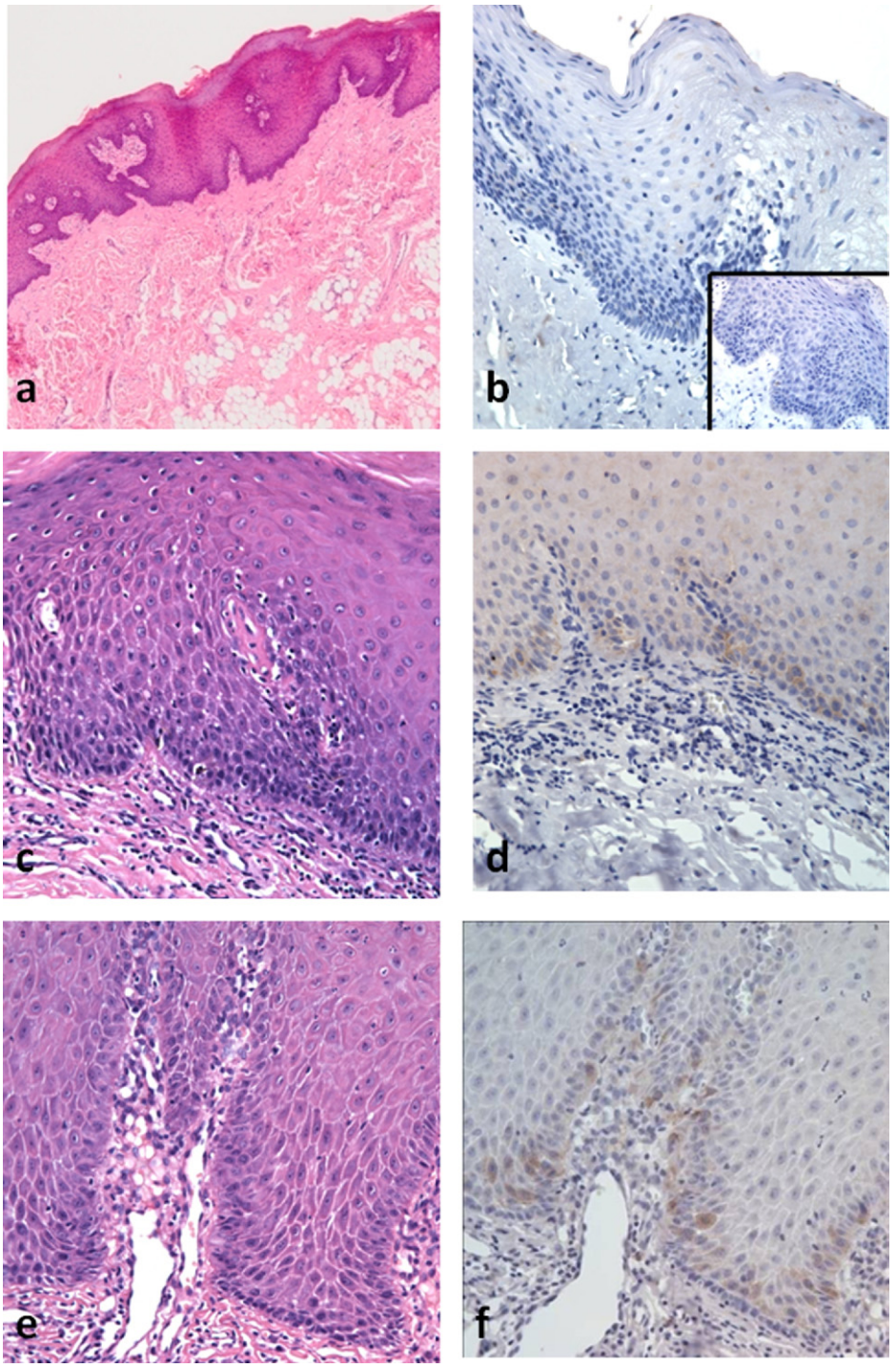

Fig. 1. Representative hematoxylin and eosin (H&E) and avidin-biotin peroxidase complex (ABC) staining of BUBR1 protein. **a,** Normal mucosa (H&E, ×40); **b,** normal mucosa revealing negative staining (ABC, ×100); negative staining has also been noted for fibrous hyperplasia (inset; ABC, ×100); **c,** hyperkeratosis/epithelial hyperplasia (H&E, ×100); **d,** hyperkeratosis/epithelial hyperplasia of **c** revealing basal layer staining (ABC, ×100); **e,** hyperkeratosis/epithelial hyperplasia (H&E, ×100); **f,** hyperkeratosis/epithelial hyperplasia of **e** revealing lower suprabasal layer staining (ABC, ×100).

also for SCC of human oral mucosa. Therefore, our findings suggest that overexpression of BUBR1 protein in oral squamous cell carcinogenesis may be associated with centrosome amplification. Our results also suggest that BUBR1 protein overexpression is an early event in human oral squamous cell carcinogenesis.

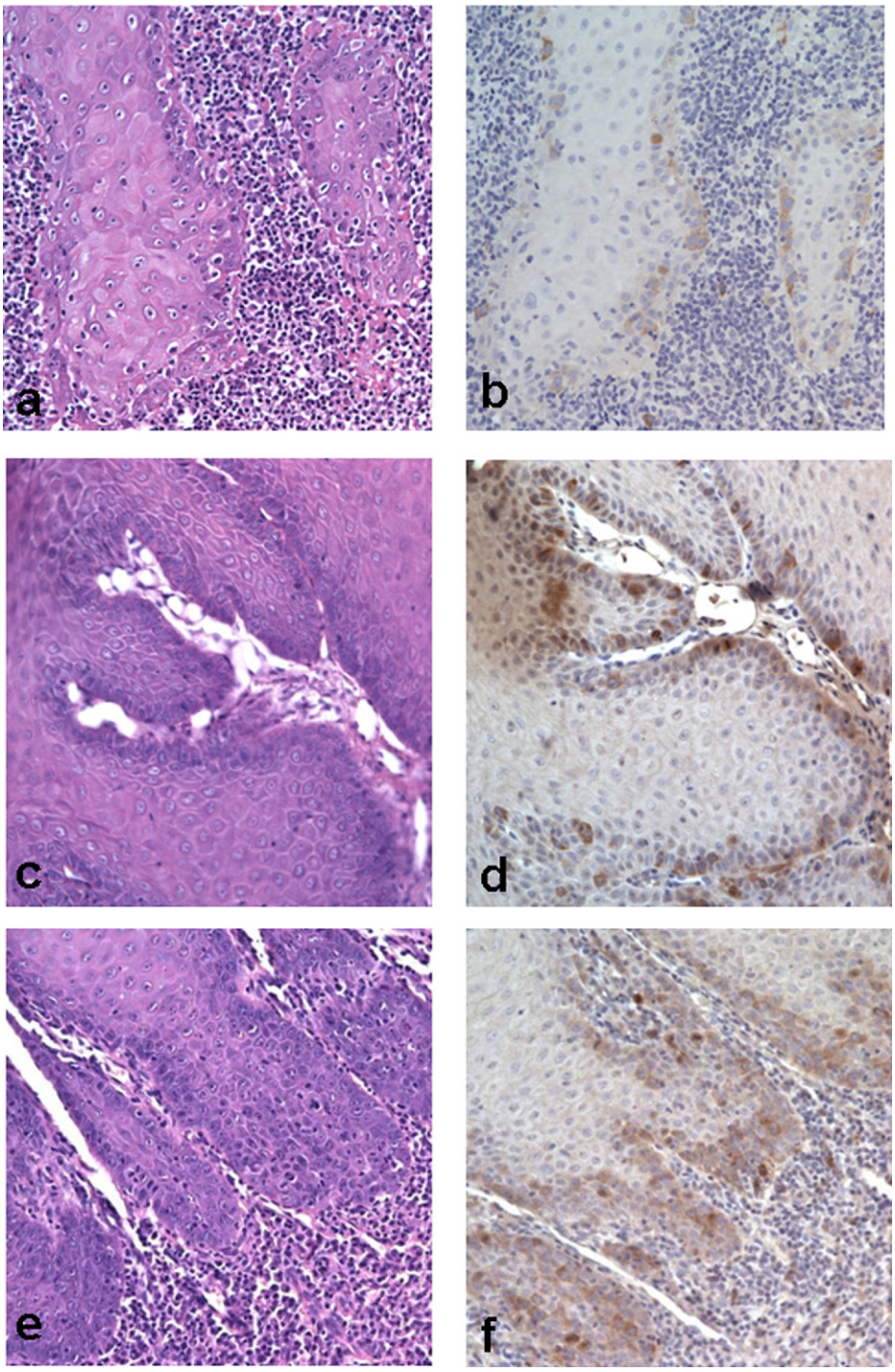

Fig. 2. Representative H&E and ABC staining of BUBR1 protein of oral epithelial dysplasia (OED). **a,** Mild OED (H&E, ×100); **b,** mild OED of **a** revealing basal layer staining (ABC, ×100); **c,** mild OED (H&E, ×100); **d,** mild OED of **c** revealing lower suprabasal layer staining (ABC, ×100); **e,** moderate OED (H&E, ×100); **f,** moderate OED of **e** revealing upper suprabasal layer staining (ABC, ×100).

LOH, defined by microsatellite markers, is commonly used in the recognition of gene loss in cancer studies. LOH analysis detects any allelic imbalance and not just allelic loss. Although allelic imbalance on chromosome 15q21.3 in head and neck SCCs and corresponding lymph node metastases has been reported,[15]

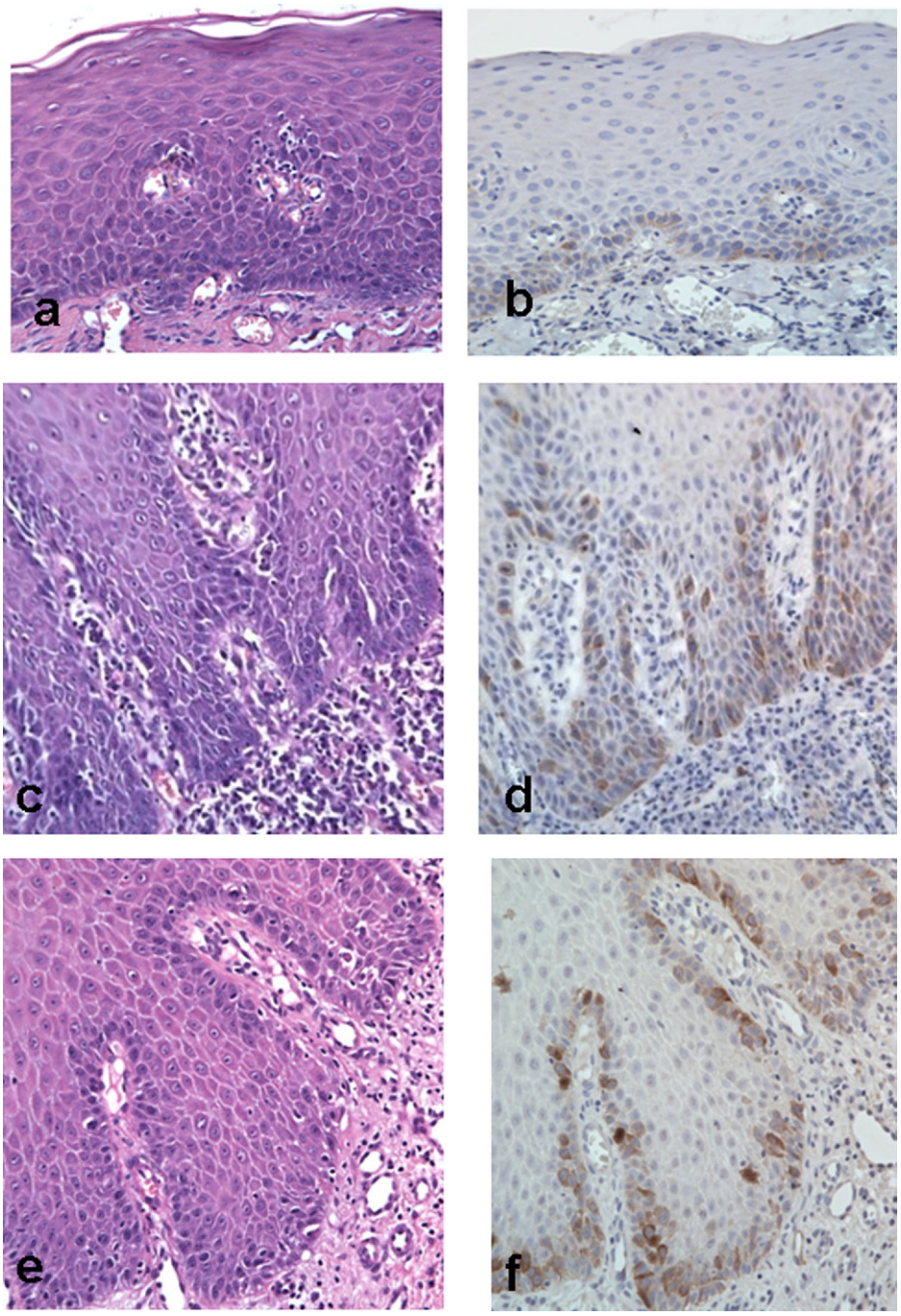

Fig. 3. Representative H&E and ABC staining of BUBR1 protein of submucous fibrosis (SF), lichen planus (LP) and verrucous hyperplasia (VH). **a,** SF (H&E, ×100); **b,** SF of **a** revealing basal layer staining (ABC, ×100); **c,** LP (H&E, ×100); **d,** LP of **c** revealing lower suprabasal layer staining (ABC, ×100); **e,** VH (H&E, ×100); **f,** VH of **e** revealing upper suprabasal layer staining (ABC, ×100).

LOH of *hBUB1B* gene (located at chromosome 15q14-21) for SCC of human oral mucosa has not yet been determined, to the best of our knowledge. Reviewing the English language literature, LOH analysis for *hBUB1B* gene in cancer research has been reported only in a study for human bladder cancers in which only one LOH of *hBUB1B* gene in the sample of 15 patients (6.7%) has been identified.[37] Therefore, the

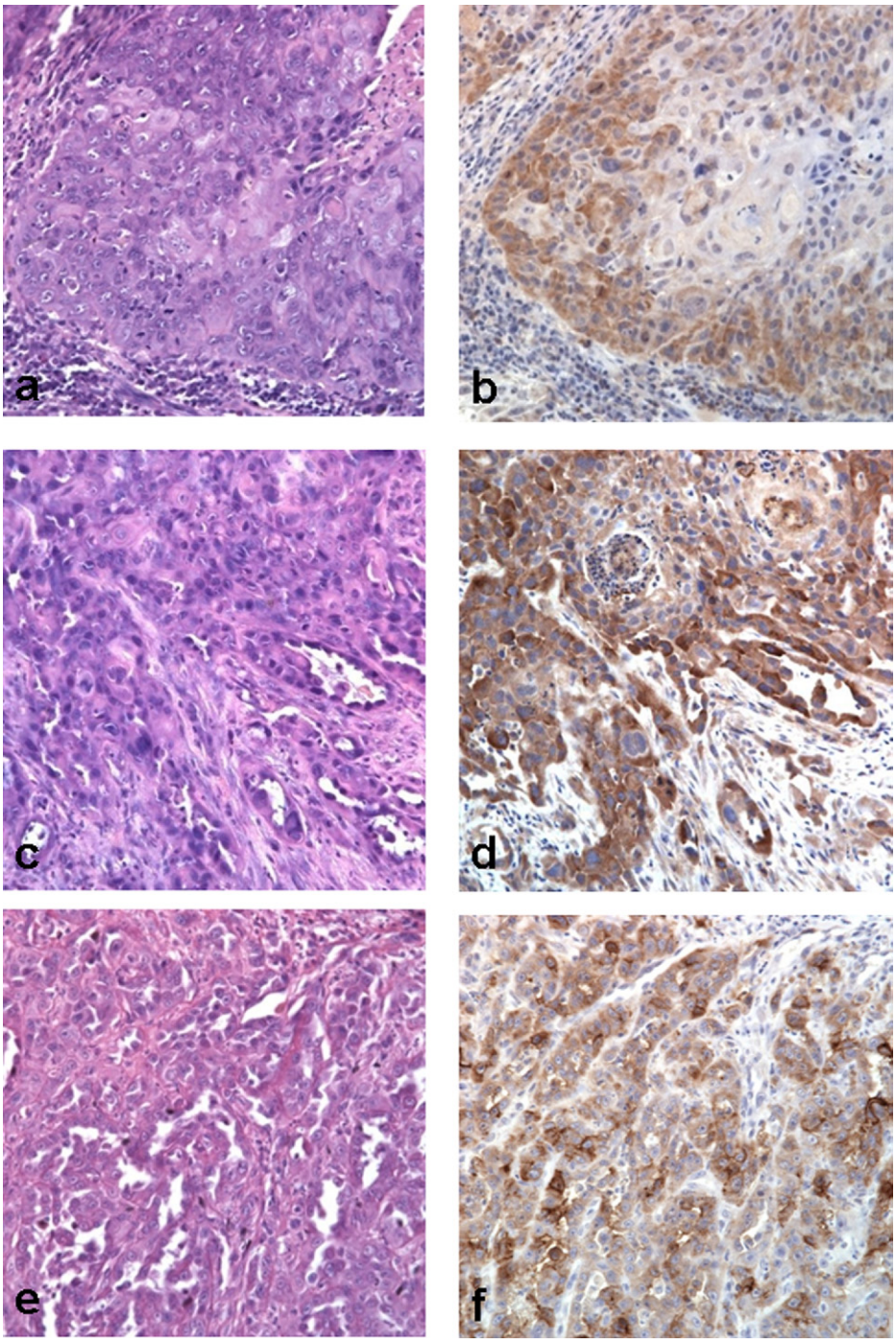

Fig. 4. Representative H&E and ABC staining of BUBR1 protein of squamous cell carcinoma (SCC) of well, moderate, and poor differentiation. **a,** Well-differentiated SCC (H&E, ×100); **b,** well-differentiated SCC of **a** (ABC, ×100); **c,** moderately differentiated SCC (H&E, ×100); **d,** moderately differentiated SCC of **c** (ABC, ×100); **e,** poorly differentiated SCC (H&E, ×100); **f,** poorly differentiated SCC of **e** (ABC, ×100).

very low number of LOH found in this cohort of bladder cancer patients[37] suggested that a gene dose effect for *hBUB1B* gene may not be a significant factor in mitotic checkpoint failure in bladder cancer. One may speculate whether a similar issue of aforementioned bladder cancer would be implicated to oral SCC. Is LOH of *hBUB1B* gene expressed only in a very small number of cases of oral SCC? More comprehensive

Table IV. Correlations of number of BUBR1-stained cells among normal, potentially malignant disorders and squamous cell carcinoma of human oral mucosa

	Normal mucosa/fibrous hyperplasia	*Epithelial dysplasia*	*Hyperkeratosis/ epithelial hyperplasia*	*Lichen planus*	*Submucous fibrosis*	*Verrucous hyperplasia*	*Squamous cell carcinoma*
Mean ± SD (%)	0.00 ± 0.00	42.85 ± 15.90	39.50 ± 20.48	45.51 ± 27.18	37.34 ± 15.07	68.84 ± 13.62	100 ± 0.00
Normal mucosa/fibrous hyperplasia		<.0001*	<.0001*	<.0001*	<.001*	<.0001*	<.0001*
Epithelial dysplasia	<.0001*		.5532	.6523	.3352	.0139*	<.0001*
Hyperkeratosis/epithelial hyperplasia	<.0001*	.5532		.2353	.6554	.0008*	<.0001*
Lichen planus	<.0001*	.6523	.2353		.1118	.0252*	<.0001*
Submucous fibrosis	<.0001*	.3352	.6554	.1118		.0002*	<.0001*
Verrucous hyperplasia	<.0001*	.0139*	.0008*	.0252*	.0002*		<.0001*
Squamous cell carcinoma	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	

*Statistically significant, Student *t* test.

studies are required to investigate this matter further. On the other hand, BUBR1 overexpression has been reported during the G2/M phase of the cell cycle.[38] This would explain why BUBR1 expression was also noted for the abnormal mitotic oral keratinocytes in our study. Consequently, there is still some controversy surrounding the issue, and although overexpression of BUBR1 protein has been found in most human cancers,[16,25,26,39,40] including oral cancer, as demonstrated in the current study, the exact regulatory pathway of BUBR1 in human carcinogenesis remains to be elucidated.

Mutation of *hBUB1B* gene appears to be a rare event in human malignancies,[41-43] supporting the view that BUBR1 overexpression is a result of the up-regulation of a normal gene. Hence, BUBR1 protein overexpression in human oral squamous cell carcinogenesis, as noted in the present study, may reflect up-regulated compensation for the loss of a normal checkpoint function. Moreover, BUBR1 overexpression in human oral squamous cell carcinogenesis is also possibly attributable to dysregulated expression of other components of the mitotic spindle-associated protein complex, such as BUB1 and BUB3. Further study of the association of other mitotic spindle-associated proteins with human oral squamous cell carcinogenesis would be worthwhile.

Although the sample size for oral epithelial dysplasia in the current study is insufficient for statistical analyses on BUBR1 expression, 9 of 10 samples of mild oral epithelial dysplasia revealed lower suprabasal layer staining, whereas the moderate oral epithelial dysplasia samples revealed only upper suprabasal layer staining. This staining pattern of upward extension of BUBR1 protein, from the lower suprabasal layers for mild oral epithelial dysplasia to the upper suprabasal layers for moderate oral epithelial dysplasia, may perhaps reflect the severity of oral epithelial dysplasia. Further study with larger sample sizes would help to investigate this interesting hypothesis.

The concept of "field cancerization" is well accepted for patients frequently contacted with carcinogens, suggesting that the entire oral mucosa is exposed to carcinogens and multiple foci of transformed tissue clones are expected in these patients of high risk for malignancy.[44] Then, the enhanced expression of BUBR1 observed in this study would be indicative of manifestation of field carcinogenesis in our cohort of patients heavily exposed to betel-quid, alcohol, and cigarettes. On the other hand, does the enhanced expression of BUBR1 noted for oral PMD in this study indicate a tendency toward malignant transformation? This question remains to be clarified only by similar studies on biopsies from patients at low risk for malignancy. All the lichen planus patients in this study did not chew betel-quid, drink alcohol, or smoke cigarettes and belong to patients with low risk for malignancy. Therefore, the enhanced BUBR1 expression would appear to be an indicative of a predilection for transformation in this category of lichen planus patients.

In conclusion, our results may suggest that BUBR1 protein is one of the contributing factors involved in the pathogenesis of oral SCC. These results also hypothesize that BUBR1 protein is a putative biomarker for human oral squamous cell carcinogenesis. However, a more precise conclusion would be acquired only with a follow-up study to compare the malignant transformation rates between those samples of BUBR1-positive oral PMDs and those of BUBR1-negative oral PMDs.

REFERENCES

1. Misra S, Chaturvedi A, Misra NC. Oral carcinoma. In: Johnson CD, Taylor I, editors. Recent advances in surgery, volume 25. London: Royal Society of Medicine Press; 2002. p. 71-86.

2. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80:827-41.
3. Isaäc van der Waal. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol 2009;45:317-23.
4. Silverman S Jr. Oral lichen planus: a potentially premalignant lesion. J Oral Maxillofac Surg 2000;58:1286-8.
5. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
6. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978;46:518-39.
7. Shear M, Pindborg JJ. Verrucous hyperplasia of the oral mucosa. Cancer 1980;46:1855-62.
8. Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. J Oral Pathol Med 2008;37:127-33.
9. Cruz I, Napier SS, van der Waal I, Snijders PJ, Walboomers JM, Lamey PJ, et al. Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. J Clin Pathol 2002;55:98-104.
10. Saunders WS, Shuster M, Huang X, Gharaibeh B, Enyenihi AH, Petersen I, et al. Chromosomal instability and cytoskeletal defects in oral cancer cells. Proc Natl Acad Sci U S A 2000;97:303-8.
11. Skoufias DA, Andreassen PR, Lacroix FB, Wilson L, Margolis RL. Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. Proc Natl Acad Sci U S A 2001;98:4492-7.
12. Minhas KM, Singh B, Jiang WW, Sidransky D, Califano JA. Spindle assembly checkpoint defects and chromosomal instability in head and neck squamous cell carcinoma. Int J Cancer 2003;107:46-52.
13. Mondal G, Sengupta S, Panda CK, Gollin SM, Saunders WS, Roychoudhury S. Overexpression of Cdc20 leads to impairment of the spindle assembly checkpoint and aneuploidization in oral cancer. Carcinogenesis 2007;28:81-92.
14. Thirthagiri E, Robinson CM, Huntley S, Davies M, Yap LF, Prime SS, et al. Spindle assembly checkpoint and centrosome abnormalities in oral cancer. Cancer Lett 2007;258:276-85.
15. Poetsch M, Kleist B. Loss of heterozygosity at 15q21.3 correlates with occurrence of metastases in head and neck cancer. Mod Pathol 2006;19:1462-9.
16. Taylor SS, Ha E, McKeon F. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. J Cell Biol 1998;142:1-11.
17. Jablonski SA, Chan GK, Cooke CA, Earnshaw WC, Yen TJ. The hBUB1 and hBUBR1 kinases sequentially assemble onto kinetochores during prophase with hBUBR1 concentrating at the kinetochore plates in mitosis. Chromosoma 1998;107:386-96.
18. Seike M, Gemma A, Hosoya Y, Hosomi Y, Okano T, Kurimoto F, et al. The promoter region of the human BUBR1 gene and its expression analysis in lung cancer. Lung Cancer 2002;38:229-34.
19. Li W, Lan Z, Wu H, Wu S, Meadows J, Chen J, et al. BUBR1 phosphorylation is regulated during mitotic checkpoint activation. Cell Growth Differ 1999;10:769-75.
20. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet 2004;36:744-9.
21. Matsumoto T, Baker DJ, d'Uscio LV, Mozammel G, Katusic ZS, van Deursen JM. Aging-associated vascular phenotype in mutant mice with low levels of BubR1. Stroke 2007;38:1050-6.
22. Rao CV, Yang YM, Swamy MV, Liu T, Fang Y, Mahmood R, et al. Colonic tumorigenesis in BubR1+/-ApcMin/+ compound mutant mice is linked to premature separation of sister chromatids and enhanced genomic instability. Proc Natl Acad Sci U S A 2005;102:4365-70.
23. Shichiri M, Yoshinaga K, Hisatomi H, Sugihara K, Hirata Y. Genetic and epigenetic inactivation of mitotic checkpoint genes hBUB1 and hBUBR1 and their relationship to survival. Cancer Res 2002;62:13-7.
24. Grabsch H, Takeno S, Parsons WJ, Pomjanski N, Boecking A, Gabbert HE, et al. Overexpression of the mitotic checkpoint genes BUB1, BUBR1, and BUB3 in gastric cancer—association with tumor cell proliferation. J Pathol 2003;200:16-22.
25. Yamamoto Y, Matsuyama H, Chochi Y, Okuda M, Kawauchi S, Inoue R, et al. Overexpression of BUBR1 is associated with chromosomal instability in bladder cancer. Cancer Genet Cytogenet 2007;174:42-7.
26. Scintu M, Vitale R, Prencipe M, Gallo AP, Bonghi L, Valori VM, et al. Genomic instability and increased expression of BUB1B and MAD2L1 genes in ductal breast carcinoma. Cancer Lett 2007;254:298-307.
27. Pinto M, Vieira J, Ribeiro FR, Soares MJ, Henrique R, Oliveira J, et al. Overexpression of the mitotic checkpoint genes BUB1 and BUBR1 is associated with genomic complexity in clear cell kidney carcinomas. Cell Oncol 2008;30:389-95.
28. Wada N, Yoshida A, Miyagi Y, Yamamoto T, Nakayama H, Suganuma N, et al. Overexpression of the mitotic spindle assembly checkpoint genes hBUB1, hBUBR1 and hMAD2 in thyroid carcinomas with aggressive nature. Anticancer Res 2008;28:139-44.
29. Pindborg JJ, Daftary DK, Mehta FS. A follow-up study of sixty-one oral dysplastic precancerous lesions in Indian villagers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1977;43:383-90.
30. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79:321-9.
31. Wright A, Shear M. Epithelial dysplasia immediately adjacent to oral squamous cell carcinoma. J Oral Pathol 1985;14:559-64.
32. Giorno R. A comparison of two immunoperoxidase staining methods based on the avidin-biotin interaction. Diagn Immunol 1984;2:161-6.
33. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643-9.
34. Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. Nature 1997;386:623-7.
35. Shin HJ, Baek KH, Jeon AH, Park MT, Lee SJ, Kang CM, et al. Dual roles of human BubR1, a mitotic checkpoint kinase, in the monitoring of chromosomal instability. Cancer Cell 2003;4:483-97.
36. Oikawa T, Okuda M, Ma Z, Goorha R, Tsujimoto H, Inokuma H, et al. Transcriptional control of BubR1 by p53 and suppression of centrosome amplification by BubR1. Mol Cell Biol 2005;25:4046-61.
37. Olesen SH, Thykjaer T, Ørntoft TF. Mitotic checkpoint genes hBUB1, hBUB1B, hBUB3 and TTK in human bladder cancer, screening for mutations and loss of heterozygosity. Carcinogenesis 2001;22:813-5.
38. Davenport JW, Fernandes ER, Harris LD, Neale GA, Goorha R. The mouse mitotic checkpoint gene bub1b, a novel bub1 family member, is expressed in a cell cycle-dependent manner. Genomics 1999;55:113-7.
39. Baker DJ, Perez-Terzic C, Jin F, Pitel K, Niederländer NJ, Jeganathan K, et al. Opposing roles for p16 Ink4a and p19 Arf in senescence and ageing caused by BubR1 insufficiency. Nat Cell Biol 2008;10:825-36.

40. Campo-Trapero J, Cano-Sanchez J, Palacios-Sanchez B, Llamas-Martinez S, Lo Muzio L, Bascones-Martinez A. Cellular senescence in oral cancer and precancer and treatment implications: a review. Acta Oncol 2008;47:1464-74.
41. Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, et al. Mutations of mitotic checkpoint genes in human cancers. Nature 1998;392:300-3.
42. Yamaguchi K, Okami K, Hibi K, Wehage SL, Jen J, Sidransky D. Mutation analysis of hBUB1 in aneuploid HNSCC and lung cancer cell lines. Cancer Lett 1999;139:183-7.
43. Hernando E, Orlow I, Liberal V, Nohales G, Benezra R, Cordon-Cardo C. Molecular analysis of the mitotic checkpoint components hsMAD2, hBUB1 and hBUB3 in human cancer. Int J Cancer 2001;95:223-7.
44. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res 2003; 63:1727-30.

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