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구강편평세포암종에서 DEK 발현에 관한 연구

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<Abstract>

Expression of DEK in Oral Squamous Cell Carcinoma

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Human DEK gene on chromosome 6p encodes a 43kD nuclear phospoprotein that was originally identified as part of a fusion protein found in a subset of acute myeloid leukemia carrying a t(6;9) translocation. Although DEK upregulation has been described in a number of human malignancies and was significantly associated with high histologic grade, lymph node metastasis and/or advanced clinical stage, no previous report has evaluated the expression of DEK protein and its clinical significance in oral squamous cell carcinoma (OSCC). Our aims were to determine DEK expression in tissue samples of normal oral mucosa and OSCC by immunohistochemistry, to analyze the correlation between DEK expression and clinicopathological parameters, and to evaluate the value of DEK as a prognostic marker for patient's survival. Ten normal oral mucosa, 10 epithelial dysplasia, and 60 OSCC samples were studied by immunohistochemistry. DEK expression tended to increase through the full thickness of epithelium in the dysplastic mucosa when compared with those in normal oral mucosa. High expression of DEK protein (score $\geq$ 2) was found in 68.3% of OSCC cases. Statistical analysis revealed that DEK overexpression in OSCC was positively correlated with high histologic grade (p=0.001), lymph node metastasis (p=0.003), and advanced clinical stage (p=0.039). In the Kaplan-Meier survival analysis, DEK overexpression was significantly associated with decreased overall survival in patients with OSCC (p=0.019). Our results suggest that DEK overexpression may be a reliable marker to predict the clinical outcome in OSCC.

Key words: DEK, Oral Squamous Cell Carcinoma

Ⅰ. INTRODUCTION

Human DEK gene on chromosome 6p encodes a 43kD nuclear phospoprotein that was originally identified as part of a fusion protein found in a subset of acute myeloid leukemia carrying a t(6;9) translocation[1)]. DEK has been found to function as an architectural protein, a majority of which is bound to chromatin, and to regulate DNA replication, transcription, DNA repair, as well as RNA splicing[2)]. According to several in vitro studies, DEK overexpression may play a role in tumor development through suppressing cellular senescence, apoptosis, and differentiation, which result in promoting cell growth and survival[3,4)].

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DEK upregulation has been described in a number of human malignancies, regardless of t(6;9) translocation, such as hepatocellular carcinoma, glioblastoma, ovarian carcinoma, breast carcinoma, cervical carcinoma, melanoma, Merkel cell carcinoma, and colorectal carcinoma[5-11]. In particular, DEK overexpression in bladder cancer and retinoblastoma has been associated with the gain of chromosome 6p22 that contains the *DEK* gene[12,13].

DEK expression has been shown to be correlated with various clinicopathological parameters in several cancers. A significant correlation of DEK expression with high histologic grade has been reported in breast, ovarian and colorectal cancer[7,9,11]. Also, DEK was significantly expressed in the tumors with lymph node metastasis and/or advanced clinical stage[9,11]. In the area of oral cancer, however, there has been only one report demonstrating upregulation of DEK in tobacco chewingmediated oral squamous cell carcinoma (OSCC)[14]. They just focused on the identification of differential expression of DEK at the mRNA level between tumor tissues and normal mucosa. To our knowledge, no previous report has evaluated the expression of DEK protein in OSCC tissue samples and its clinicopathological significance. Therefore, our aims in the present study were to determine DEK expression in tissue samples of normal oral mucosa, epithelial dysplasia, and OSCC by immunohistochemistry, to analyze the correlation between DEK expression and clinicopathologic parameters, and to evaluate the value of DEK as a prognostic marker for patient's survival.

II. MATERIALS AND METHODS

1. Patients and tissue samples

Ten normal oral mucosa samples, 10 epithelial dysplasia, and 60 OSCC samples were studied by immunohistochemistry. All tumors were surgically removed at the Department of Oral and Maxillofacial Surgery, Seoul National University Dental Hospital. Of the 60 OSCC patients, 49 were male, and the mean patient age was 56 years (ranged from 21 to 86 years). The patients'clinical information, including age, gender, tumor size, lymph node metastasis, recurrence, and TNM stage, is summarized in Table 1. Tumors were staged according to the TNM classification as recommended by the American Joint Committee on Cancer (AJCC).

2. Immunohistochemistry

Formalin-fixed, paraffin-embedded sections (4μm) were used for immunohistochemical staining. The sections were

Table 1. Clinicopathological characteristics of 60 patients with OSCC

variable	Number of cases
Age (mean)	56 years
range	21-86 years
Gender	
male	49
female	11
pT	
T1	10
T2	27
T3	2
T4	21
Differentiation	
well	40
moderately/poorly	20
pN	
N0	21
N1	18
N2	21
Clinical Stage	
I	6
II	6
III	13
IV	35

deparaffinized through a series of xylene baths and then rehydrated in graded alcohols. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for 10 minutes at room temperature. For antigen retrieval, the sections were treated with target retrieval solution, pH 9.0 (DAKO, Glostrup, Denmark) in the microwave for 10 minutes. Sections were incubated in 10% normal goat serum for 30 minutes to reduce non-specific staining. Sections were then incubated with a rabbit polyclonal anti-human DEK antibody (1:200, ab22826; Abcam, Cambridge, UK) for 1 hour at room. The slides were stained using the DAKO EnVision kit. Immunohistochemical reactions were developed with diaminobenzidine (DAB) as the chromogenic peroxidase substrate, and slides were counterstained with Mayer's hematoxylin. Breast cancer tissue was used as a positive control. As a negative control, the primary antibody was replaced with PBS.

3. Evaluation of immunohistochemical staining

DEK expression was evaluated independently by two investigators who scored staining using semiquantitive immunoreactive scores. Only the nuclear expression was considered as positive staining. The immunostaining for DEK was scored as 0 (no or < 5% positive cells), 1 (5-25% positive cells), 2 (26-50% positive cells) or 3 (>50% positive cells)[8]. Cases with a score ≥ 2 were defined as high expression, whereas cases with a score less than 2 were interpreted as low expression.

4. Western blot analysis

A total of 13 OSCC cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM), a mixture of DMEM and F12 or RPMI 1640, supplemented with 10% FBS and 1% antibiotic antimycotics. All cell lines were cultured at 37℃ in a humidified atmosphere of 5% CO_2. Whole cell lysates were harvested with RIPA buffer supplemented with protease inhibitor cocktail (Sigma-Aldrich, MO, USA). The BCA protein assay kit (Pierce Biotechnology, Rockford, USA) was used to determine the concentration of protein. Samples (30μg) were mixed with sample buffer, incubated at 95℃ for 5min, and electrophoresed on 12% SDS/ polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes (Pall, MI, USA) and probed with antibody to DEK (1:1000, BD Biosciences, San Jose, CA).

5. Statistical analysis

All of the statistical analyses were performed using the IBM SPSS statistics 20 (IBM Corporation, NY, USA). Correlation between DEK expression and clinicopathological parameters were analyzed by chi-square test and Fisher's exact tests. The survival rates were calculated using the Kaplan- Meier method and analyzed using the log-rank test. $P<0.05$ was considered statistically significant.

Ⅲ. RESULTS

1. DEK protein expression in normal oral mucosa, epithelial dysplasia, and OSCC tissue samples

Nuclear expression of DEK protein was detected in both normal oral mucosa and OSCC tissues. In the normal oral mucosa, DEK expression was detected mainly in the nuclei of the basal and parabasal layers of epithelium (Fig. 1A). However, DEK was more diffusely expressed up to the superficial layer of epithelium in the epithelial dysplasia (Fig. 1B). Significant increase of DEK expression was found in early invasive tumor area when compared to the adjacent dysplastic epithelium (Fig. 1C). 68.3% of OSCC cases showed high expression (score ≥ 2) of DEK protein (Fig. 1D).

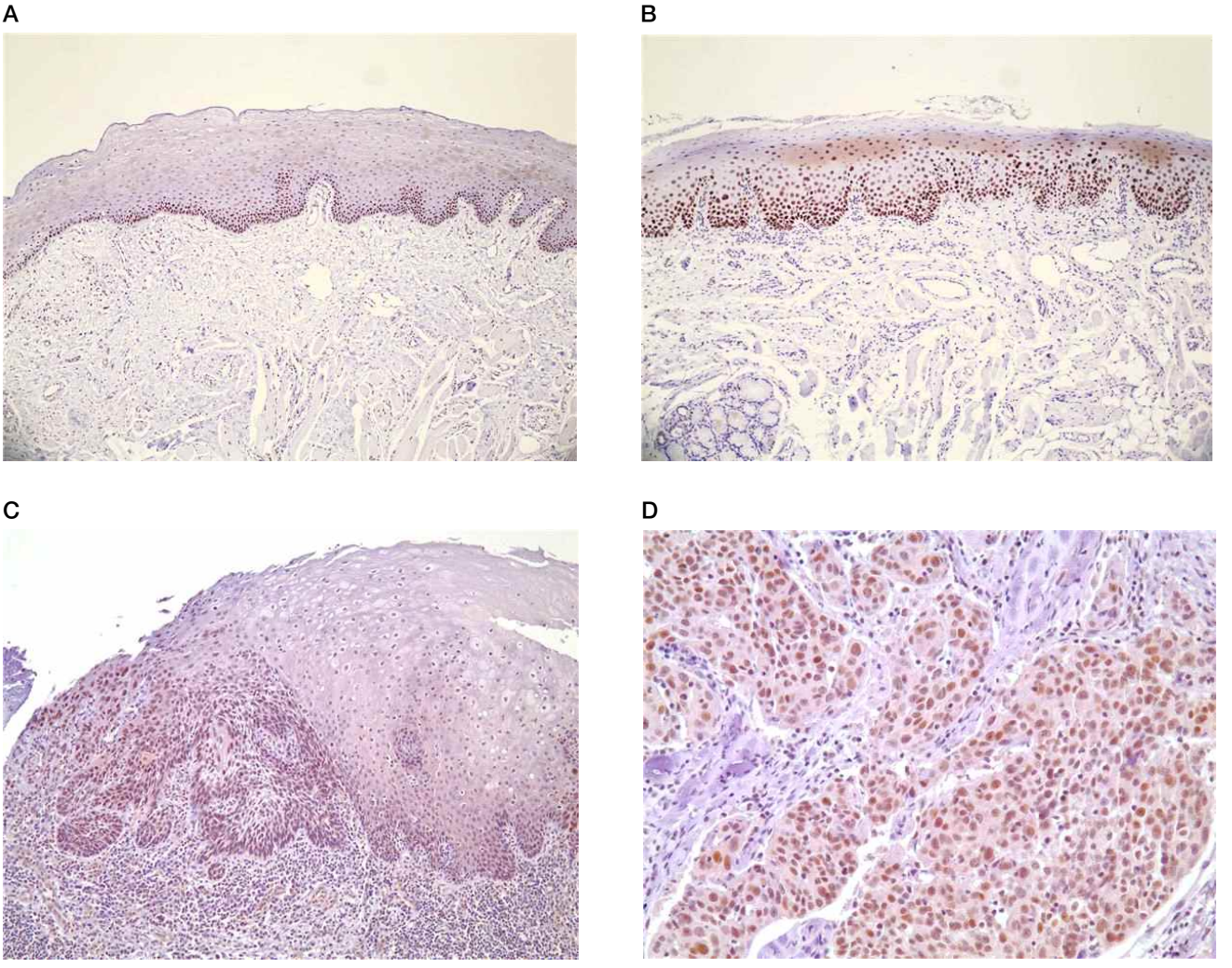

Fig. 1. Immunohistochemical staining of DEK protein. (A) DEK was expressed mainly in the nuclei of the basal and parabasal layers of epithelium in the normal mucosa (x100). (B) DEK expression was increased up to the superficial layer of epithelium in the dysplastic mucosa (x100). (C) Early invasive carcinoma area showed more increased expression of DEK when compared to the adjacent dysplastic epithelium (x100). (D) Strong expression of DEK in OSCC (x200).

2. Correlation between DEK expression and the clinicopathological parameters of OSCC

Table 2 shows the correlations between DEK expression and clinicopathological parameters. Statistical analysis revealed that DEK overexpression in OSCC was positively correlated with high histologic grade, presence of lymph node metastasis, and advanced clinical stage. DEK expression was more highly expressed in moderately and poorly differentiated OSCC than in well-differentiated cases (P=0.001). Also, high expression of DEK protein was more frequently detected in the OSCC samples with lymph node metastasis (P=0.003) and advanced clinical stage (P=0.039). However, there was no correlation between DEK expression and age, gender, tumor size, or recurrence.

In the Kaplan-Meier survival analysis, there was significant difference in overall survival between the patients with high and low expression of DEK (P=0.019, Fig. 2)

Table 2. Correlation between DEK expression and clinicopathological parameters in OSCC.

Variables	cases (%)	DEK expression		P-value
		Low (%)	High (%)	
	n= 60	n = 19	n = 41	
Gender				
Male	49 (81.7)	18 (36.7)	31 (63.3)	0.148
Female	11 (18.3)	1 (9.1)	10 (90.9)	
Age				
〈 56	24 (40.0)	7 (29.2)	17 (70.8)	0.784
≥ 56	36 (60.0)	12 (33.3)	24 (66.7)	
Tumor size				
T1+T2	37 (61.7)	12 (32.4)	25 (67.6)	1.000
T3+T4	23 (38.3)	7 (30.4)	16 (69.6)	
Differentiation				
Well	40 (66.7)	18 (45.0)	22 (55.0)	0.001
Moderately/poorly	20 (33.3)	1 (5.0)	19 (95.0)	
LN metastasis				
Negative	21 (35.0)	12 (57.1)	9 (42.9)	0.003
Positive	39 (65.0)	7 (17.9)	32 (82.1)	
Recurrence				
No	44 (73.3)	15 (34.1)	29 (65.9)	0.754
Yes	16 (26.7)	4 (25.0)	12 (75.0)	
TNM stage				
I+II	12 (20.0)	7 (58.3)	5 (41.7)	0.039
III+IV	48 (80.0)	12 (25.0)	36 (75.0)	

3. Expression analysis of DEK in OSCC cell lines

Expression of DEK protein was evaluated in 13 OSCC cell lines by western blot analysis. All cell lines expressed DEK protein to varying degree. Relatively strong expression of DEK was detected in HSC-3 and Ca9-22 cell lines (Fig. 3).

IV. DISCUSSION

Oral cancer is the eleventh most common cancer in the world, with an estimated 300,000 new cases and 145,000 deaths annually[15]. The majority of oral cancers are squamous cell carcinoma. Despite of advances in understanding of the underlying molecular pathogenesis of OSCC, it remains a lethal disease in over 50% of the cases because of late detection of advanced stage cancer. Therefore, developing possible new therapeutic molecular targets is of importance to improve patient's prognosis.

Many researches have supported that DEK is a bona fide oncogene that play a role in central pathway to promote tumor growth and survival. Although DEK upregulation has been described in a number of human malignancies,

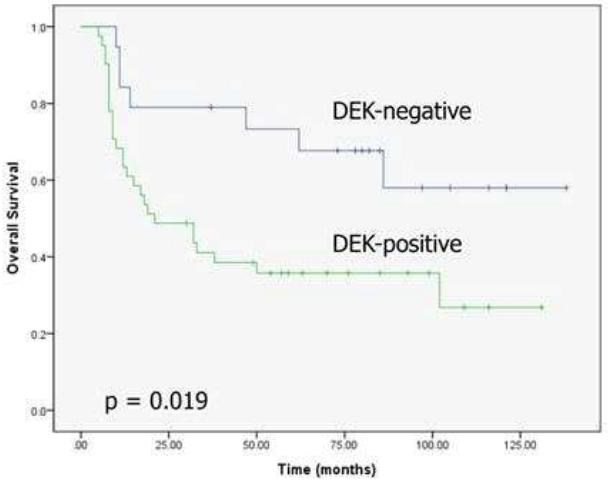

Fig. 2. Kaplan–Meier analysis of overall survival rate in 60 OSCC patients according to DEK expression (Log–rank test).

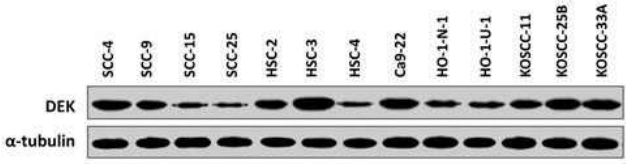

Fig. 3. Expression levels of DEK were examined in 13 OSCC cell lines by western blot analysis.

oncogenic DEK activities have been more elucidated by Wise–Draper and his colleagues. They demonstrated that DEK overexpression promotes epithelial transformation and DEK knock mice could not form benign papilloma in a classical chemical carcinogenesis model[4]. These results support the oncogenic abilities of DEK more directly, in addition to their previous findings demonstrating that DEK suppresses senescence, apoptosis, and epithelial differentiation[2–4]. In their report, DEK depletion by RNA interference resulted in apoptosis of cancer cells both in vivo and in vitro[4], suggesting that targeted suppression of DEK might be a possible new treatment strategy to suppress cellular transformation and induce apoptosis of tumor cells in human cancer.

In the present study, DEK expression tended to increase through the full thickness of epithelium in the dysplastic oral mucosa when compared with normal epithelium which showed positivity restricted to basal and parabasal layers. In addition, significant increase of DEK expression was found in early invasive tumor area compared to the adjacent dysplastic epithelium. Similar results are also found in cervical, colorectal, and gastric cancer[8,11,16]. DEK expression in dysplastic lesion and cancer was significantly higher than that in the adjacent normal mucosal of cervix, colon, and stomach. Also, DEK expression was found to be a tumor marker that can distinguish benign nevi from malignant melanoma[17]. These results suggest that DEK may play a role in the early stage of carcinogenesis in several types of organs. Therefore, it is needed to qualify the value of DEK expression as a biomarker for early diagnosis of OSCC.

DEK expression has been associated with several clinicpathological parameters in diverse malignancies. In consistent with our result, a significant correlation of DEK expression with high histologic grade was found in breast, cervical, ovarian, colorectal, and gastric cancer[7–9,11,16]. It means that DEK expression could aid in gauging the differentiation potential of tumor cells. In line with the previous results in breast, colon, and stomach[9,11,16], DEK was significantly expressed in OSCC with lymph node metastasis and advanced clinical stage. In addition, we found that patient with high DEK expression had worse survival rate than those with low expression as like other reports in colorectal and gastric cancers[11,16]. Therefore, DEK expression may be also a reliable biomarker to predict the prognosis in OSCC.

Despite the results showing strong association between DEK expression and cancer, molecular mechanisms underlying tumor progression by DEK are still unclear. It was known that DEK is an inhibitor of p53–dependent and –independent cellular senescence and apoptosis[2,4], and transcriptionally upregulated by the p16–pRb–E2F pathway[18].

Recently, Lin et al. studied the mechanism by which DEK overexpression regulate cancer growth and apoptosis in colorectal cancer[19]. They demonstrated that upregulation of DEK was involved in the p53/MDM, Bcl-2/Bax, and caspase pathway. When the expression of DEK was suppressed, the expression levels of mutant-p53 and MDM2 decreased greatly and Bcl-2/Bax ratio was also significantly reduced so that it could induce the activation of caspase pathway for apoptosis. Taken together, DEK could play a role in tumor progression by inhibiting cellular senescence, enhancing the proliferation, and inhibiting the apoptosis of cancer cells using those signaling pathways. Further investigation is needed to identify another molecular pathway to control the oncogenic role of DEK.

In conclusion, we investigated the expression and clinopathological significance of DEK in OSCC. Significant DEK expression was found in OSCC tissue samples and then high expression of DEK significantly correlated with the histologic differentiation, LN metastasis, and clinical stage. Patients with high expression of DEK showed worse overall survival rate than those with low expression. Our results suggest that DEK may play a role in tumor progression of OSCC and could be a possible prognostic factor for patient with OSCC.

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