DEK: A novel early screening and prognostic marker for breast cancer

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Abstract. The present study aimed to investigate the expression status and clinical implications of DEK in breast cancer, in order to contribute to developments in breast cancer management. DEK expression status was detected in 628 breast cancer specimens by western blot analysis and immunohistochemistry staining, and the correlation between DEK protein and clinico-pathological parameters and prognosis of breast cancer was subsequently determined. In comparison to para-carcinoma tissues, DEK protein was highly expressed in breast cancer specimens and was correlated with chemotherapy resistance. In total, 61.94% (389/628) of breast cancer cases exhibited high expression of DEK. According to universal analysis, it was observed that age, tumor size, histological grade, metastatic nodes and distant metastasis (P=0.024, 0.001, 0.001, 0.001 and 0.001 respectively) are key factors associated with DEK. Furthermore, compared with samples with no or low DEK protein expression, high DEK expression resulted in a significantly increased distant metastasis rate and poor disease-specific survival (P=0.001). In addition, DEK protein was detected as an independent prognostic factor (P=0.001) in the Cox regression analysis. DEK was correlated with chemotherapy resistance and may be an independent prognostic factor for breast cancer, as well as a potential therapeutic target.

Introduction

According to the World Health Organization, the number of individuals diagnosed with cancer annually has reached >1.2 million individuals, and breast cancer is responsible for 3% of female fatalities (1,2). Although a number of identified molecules are involved in the way breast cancers progress and metastasize, the mechanisms of breast cancer remain to be identified (3,4). Surgery is mainly used as a primary treatment, and chemotherapy, radiotherapy and endocrine therapy are then directed at eliminating the residual tumor cells, thus reducing the recurrence and metastasis risk. However, there remain cases of relapse or metastases in certain patients. At this point, few molecules exhibit high efficiency in predicting chemotherapy sensitivity and postoperative distant metastasis for breast cancer.

DEK, a non-histone nuclear phosphoprotein initially identified as a putative proto-oncogene, has recently been found to be associated with the regulation of hematopoiesis (5). Studies have demonstrated that not only is it associated with chromatin reconstruction and gene transcription, but it also contributes to cell apoptosis (5-7). Thus, there is a direct correlation between high expression levels of the human DEK and numerous types of human malignancy (5). Following investigation of the DEK expression level in chronic lymphocytic leukemia, Wang *et al* (6) observed a marked increase of DEK mRNA expression in patients with chronic lymphocytic leukemia, which may be useful to assess the prognosis in patients with chronic lymphocytic leukemia. At present, the DEK expression status and the clinical implication in breast cancer remain unclear. Thus, the present study aimed to investigate the expression status of DEK in breast cancer and the clinical implications in order to aid in the development of breast cancer management.

Patients and methods

Patients and tissue specimens. Patients (n=628) with histologically confirmed breast cancer who underwent radical surgery between January 2001 and January 2010 in Harbin Medical University (Nangang, China) were enrolled in the present study. Samples were obtained for immunohistochemical staining as well as prognostic analysis. The mean age of the patients was 47.28±9.43 years (range, 27-78 years). Patients underwent curative surgery, the resected specimens were pathologically examined and >10 lymph nodes were pathologically examined following surgery. Complete medical records including the ER, PR, Her2, p53 and Ki67 status were available. The study protocol was approved by Harbin Medical University. Patients were informed of the details of the study and agreed to participate.

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Western blot analysis. For western blot analysis, cells were lysed with buffer (0.1% SDS, 50 mmol/l Tris-HCl, pH 7.6; 1% NP-40, 150 mmol/l NaCl, 2 mg/ml aprotinin, 2 mg/ml leupeptin and 7 mg/ml PMSF). The protein concentrations were determined using the bicinchoninic acid Protein Assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Proteins (30 μg) were separated on 10% SDS-PAGE gels (Varsal Instruments, Beijing, China) and transferred to a polyvinylidene difluoride membrane (Varsal Instruments). After blocking, the membrane was incubated with an anti-DEK antibody (cat. no. ab166624; 1:1,000; Abcam, Cambridge, MA, USA) at 4˚C overnight. After washing, the membrane was incubated with a secondary antibody (cat. no. ZB-2301; Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd., Beijing, China) at a dilution 1:3,000 at room temperature for 1 h. Proteins were detected with an enhanced chemiluminescent kit (Varsal Instruments) and anti-β-actin antibody (cat. no. SAB5500001; 1:1,000; Sigma-Aldrich, St. Louis, MO, USA) was used as loading control. Densitometry was performed using Gel-pro Analyzer 4.0 (Media Cybernetics, Silver Spring, MD, USA).

Immunohistochemistry procedures. Thin slices of tumor tissue from all cases were fixed in 4% formaldehyde solution (pH 7.0) for periods not exceeding 24 h. Paraffin embedding was conducted, and 4 μm-thick sections were cut and placed on glass slides coated with 3-aminopropyl triethoxysilane (Seebio Biotech Inc., Shanghai, China) for immunohistochemistry. Tissue samples were stained with hematoxylin and eosin to determine histological type and grade of tumors.

Briefly, breast tumor tissues were cut at a thickness of 4 μm using a cryostat. The sections were mounted on microscope slides, air-dried and then fixed in a mixture of 50% acetone and 50% methanol. The sections were then de-waxed with xylene, gradually hydrated with gradient alcohol, and washed with phosphate-buffered saline (PBS). Sections were incubated for 60 min with the primary antibody. Following washing with PBS, sections were incubated for 30 min with the secondary biotinylated antibody (Multilink Swine anti-goat/mouse/rabbit immunoglobulin; Dako Inc., Carpinteria, CA, USA). Following washing, Avidin Biotin Complex (1:1,000 dilution, Vector Laboratories Ltd., Burlingame, CA, USA) was then applied to the sections for 30-60 min at room temperature. The immunoreactive products were visualized by catalysis of 3, 3-diaminobenzidine (DAB) by horseradish peroxidase in the presence of H_2O_2, following extensive washings. Sections were then counterstained in Gill's hematoxylin and dehydrated in ascending grades of methanol prior to clearing in xylene, and mounting under a coverslip. The sections were observed under an Olympus CX31 microscope (Olympus, Tokyo, Japan).

To score DEK as immunopositive staining, the positive cells are shown as a yellow to brown color in the nucleus and/or cytoplasm. DEK expression was classified semi-quantitatively according to the following criteria: -, <1% of neoplastic cells discretely expressed DEK; +, ≥1 of morphologically unequivocal neoplastic cells discretely expressed DEK.

Statistical analysis. All data were analyzed with SPSS statistics software (Version 13.0, SPSS Inc., Chicago, IL, USA). Correlations between DEK and other parameters were investigated using the χ^2 test, Fisher's exact test or independent t-tests. The Kaplan-Meier method was adopted to analyze disease-specific survival, while the log-rank test was used to analyze survival differences. Multivariate analysis was performed using the Cox proportional hazards model selected in forward stepwise. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Association between DEK expression and clinicopathological characteristics of breast cancer. Immunohistochemical examination showed that DEK was located in the nucleus and/or cytoplasm of breast cancer cells. It was observed that expression of DEK protein was significantly higher in breast cancer tissues compared with paracancerous tissue (61.94% vs. 6.53%; Fig. 1). Western blot analysis showed that DEK protein was significantly highly expressed in breast cancer tissues with lymph node metastasis compared with those without (P=0.001; Fig. 2). After universal analysis, DEK was observed to be correlated with age, tumor size, histological type, lymph node metastasis and distant metastasis (P=0.024, 0.001, 0.001, 0.001 and 0.001, respectively; Table I).

Association between DEK expression and the post-operative recurrence and chemotherapeutic resistance. Patients with high expression of DEK were shown to have a significantly increased distant metastasis rate. Furthermore, 185 (74.30%) of 249 breast cancers with distant metastasis exhibited DEK expression compared with 204 (53.83%) of 379 cases of non-distant metastasis (P=0.001).

The factors associated with post-operative distant metastasis with multiple analyses were also investigated. Age, tumor size, histological type, triple negative subtype and DEK expression were found to be associated with post-operative distant metastasis in breast cancer (Table II). In addition, the present study investigated the correlation between DEK expression and chemotherapeutic sensitivity in 107 patients with breast cancer who underwent neoadjuvant chemotherapy. DEK expression was expressed in 78.57, 67.86, 46.30 and 18.18% of patients with complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) (P=0.006; Table III).

Prognostic analysis. Furthermore, DEK along with age, histological type, lymph node metastasis, affected survival rate in triple-negative breast cancer. Patients with triple-negative breast cancer and high DEK expression exhibited a poorer disease-specific survival compared with those with none or low expressed DEK protein (P=0.001; Fig. 3). In the Cox regression test, DEK protein was detected as an independent prognostic factor (P=0.001; Table IV).

Discussion

DEK is a chromatin-associated oncogene whose expression has been linked to cancer through multiple mechanisms, including β-catenin activity. Recently, Privette reported that DEK is a downstream target of Ron receptor activation in murine and human models (7). The absence of DEK in the MMTV-Ron mouse model led to a significant delay in tumor development, characterized by decreased cell proliferation, diminished

Table I. Correlation between DEK expression and clinico-pathological features of breast cancer (n=628).

Variable	n	DEK$^-$	DEK$^+$	χ^2	P-value
Age, years				5.093	0.024
<40	129	38	91		
>40	499	201	298		
Tumor size				97.559	0.001
T1	126	86	40		
T2	463	105	358		
T3	27	6	21		
T4	12	2	10		
Histological grade				46.489	0.001
I	51	38	13		
II	415	165	250		
III	162	36	126		
Metastatic nodes				40.896	0.001
Negative	304	158	146		
Positive	324	91	243		
Distant metastasis				26.714	0.001
Negative	379	175	204		
Positive	249	64	185		
Triple-negative breast cancer				0.203	0.653
Yes	140	51	89		
No	488	188	300		

Table II. Multivariate analysis of the factors associated with post-operative distant metastasis.

Characteristic	Exp (B)	95% CI for Exp (B)	P-value
Age	2.847	1.302-4.116	0.010
Tumor size	1.641	1.386-2.140	0.032
Histological type	2.056	1.749-3.203	0.020
Triple-negative breast cancer	3.821	2.542-5.075	0.001
DEK	3.425	2.582-4.169	0.001
Constant	0.002		

Constant refers to the constant interest rate. CI, confidence interval.

Table III. Correlations between DEK expression and chemotherapeutic resistance in breast cancers [n=107; n (%)].

Response	n	DEK$^-$	DEK$^+$	χ^2-value	P-value
CR	11	9	2 (18.18)	12.489	0.006
PR	54	29	25 (46.30)		
SD	28	9	19 (67.86)		
PD	14	3	11 (78.57)		

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

metastasis and a decline in the number of cells expressing breast cancer stem cell markers. Overexpression of DEK was sufficient to promote cellular growth and invasion in cell lines established from MMTV-Ron mouse models (7). In another

Table IV. Cox model regression analysis of the breast cancer prognostic factors.

Variable	OR	95% CI for OR	P-value
Age	1.402	1.063-2.614	0.004
Tumor size	1.871	1.365-3.163	0.001
Histological type	1.329	1.163-1.981	0.002
Lymph node metastasis	2.132	1.655-2.806	0.001
Triple-negative breast cancer	3.284	2.749-4.157	0.001
DEK	2.776	1.923-3.260	0.001

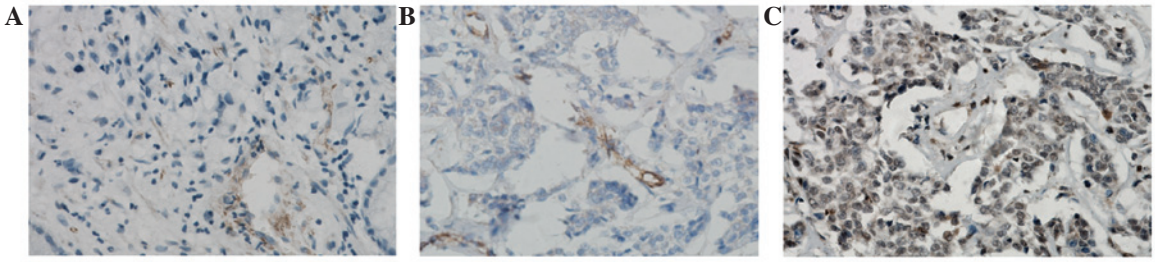

Figure 1. DEK protein was highly expressed in breast cancer tissues. Immunohistochemical staining of (A) DEK in adjacent normal tissues, x400 magnification; and (B) negative expression, x400 magnification; and (C) positive expression, x400 magnification in breast cancer.

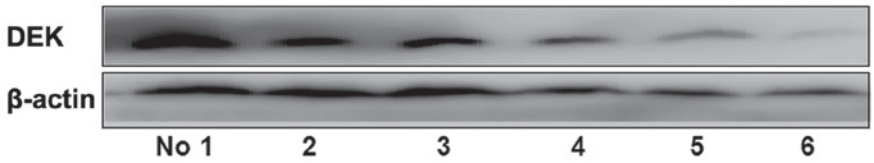

Figure 2. Western blot analysis of the level of DEK expression. DEK showed higher expression in breast cancer with lymph node metastasis (lanes, 1-3) than in non-lymph node metastasis (lanes, 4-6) (P=0.01).

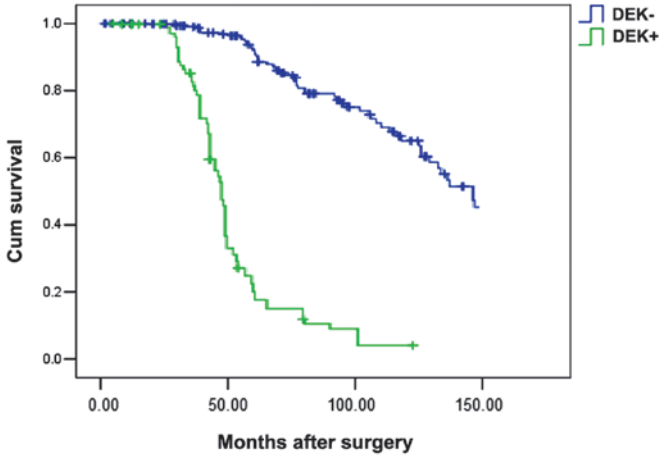

Figure 3. Overall survival curves according to DEK expression. Kaplan-Meier estimates of 5-year cumulative death rates for patients with breast cancer according to DEK expression status (P=0.001, log-rank test).

recent study based on head and neck squamous cell carcinoma (HNSCC), Adams *et al* (8) reported that DEK is required for optimal proliferation of E7-transgenic epidermal cells and for the growth of HNSCC tumors. Notably, DEK protein is universally upregulated in HPV-positive and -negative human HNSCC tumors relative to adjacent normal tissue. Furthermore, DEK knockdown inhibited the proliferation of HPV-positive and -negative HNSCC cells, establishing a functional role for DEK in human disease (8). DEK is also found to be related to the poor prognosis of gastric cancer and colorectal cancer (9,10). Thus, DEK may exhibit potential as a breast cancer treatment target. At present, the expression status of DEK protein in breast cancer and its correlation with the biological behavior of breast cancer remains unclear. Furthermore, studies addressing the association between DEK and chemotherapy sensitivity and prognosis of breast cancer are limited.

In the present study, the correlation between DEK expression and the biological behavior and clinicopathological characteristics of breast cancer was investigated. DEK protein expression was observed to be significantly higher in cancerous tissues than adjacent-tumor tissues. Furthermore, DEK protein was found to be related to tumor size, histological type, lymph node metastasis and post-operative distant metastasis in the 628 breast cancers. Following further investigation of the association between DEK expression and chemotherapeutic sensitivity, it was found that DEK expression was significantly correlated with poor chemotherapy response in breast neoadjuvant chemotherapy.

After survival analysis, cases with high-level DEK expression were significantly more likely to develop post-operative distant metastasis and exhibit poor postoperative disease specific survival. Cox regression analysis showed DEK protein was detected as an independent prognostic factor. The outcomes suggested that DEK expression has been shown to be associated with poor breast cancer prognosis. DEK may be involved in breast cancer oncogenesis and may be a potential biomarker for the metastasis and chemotherapy resistance of breast cancer.

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