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Original Article

Expression of NF-κB-inducing kinase in breast carcinoma tissue and its clinical significance

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Abstract: Objective: To investigate the expression of nuclear factor-κB-inducing kinase (NIK) in breast carcinoma tissue and tumor-adjacent normal breast tissue and evaluate its clinical significance. Methods: Surgically resected tissue specimens were collected from 82 patients with breast carcinoma who underwent surgical treatment at our hospital from March 2001 to December 2009. The diagnoses of all patients were confirmed by postoperative pathological examinations. NIK protein expression in breast carcinoma tissue and adjacent normal breast tissue was detected by immunohistochemistry; the association between NIK expression and the clinicopathological features and prognosis of patients with breast carcinoma was examined. Results: The positive expression rate of NIK in breast carcinoma tissue was significantly higher than that in normal tissue (63.4% vs. 25.6%, $P < 0.05$). Additionally, NIK expression showed no relationship to the tumor size, age, degree of differentiation, or pathological type; however, it showed a significant correlation with lymph node metastasis and the clinical stage of patients ($P < 0.05$). The five-year survival rate was significantly lower in breast carcinoma patients who were positive for NIK expression than in those who were negative for NIK expression ($P = 0.006$). Conclusion: NIK expression was significantly increased in the tumor tissue of patients with breast carcinoma, which may be an important factor that affects the prognosis of these patients.

Keywords: Breast carcinoma, NF-κB-inducing kinase

Introduction

Breast carcinoma is a type of malignant tumor that threatens the lives and health of women, and it shows a high incidence worldwide. The incidence and mortality of breast carcinoma in Chinese women are relatively low compared to the global level; however, its incidence has progressively increased in China in recent years [1]. Currently, the treatment for breast carcinoma mainly relies on comprehensive therapy, in which surgery is supplemented by radiochemotherapy and biotherapy. Recently, molecular targeted therapy has emerged as a focus of research on the treatment of breast carcinomas [2]. Although extensive research has been conducted to understand the development and progression of breast carcinomas, the exact mechanism has not been fully elucidated [3]. Presently, no targeted therapeutic drugs are available for the clinical treatment of patients with breast carcinoma. Therefore, in-depth studies of the molecular pathological mechanisms underlying the development and progression of breast carcinoma and the search for efficient therapeutic targets are of great importance for improving the clinical prognosis of patients with breast carcinoma.

Numerous studies have shown that nuclear factor-κB (NF-κB) plays an important role in the development and progression of breast carcinoma [4]. However, few studies have investigated the expression of NF-κB-inducing kinase (NIK) in breast carcinoma tissue. In this study, NIK expression in tumor tissue and tumor-adjacent normal breast tissue of patients with breast carcinoma was detected by immunohistochemical (IHC) assays to provide new ideas and methods for clinical treatment of this disease.

Materials and methods

General data collection

This study included 82 patients with breast carcinoma who underwent surgical treatment at our hospital between March 2001 and December 2009. The diagnoses of all patients were confirmed by postoperative pathological examinations. Furthermore, samples of the tumor-adjacent normal breast tissue (referred to as normal tissue) of the 82 patients were used as controls. This study was approved by the Hospital Ethics Committee, and all patients signed an informed consent form before surgery. General clinical data were collected from the patients, including name, age, pathological staging, results of preoperative and postoperative examination, and treatment outcomes. Subjects who had received radiotherapy and/or chemotherapy before surgery were excluded. All patients were followed-up by telephone calls or hospital visits every two months. The followed-up period was five years.

IHC staining

Freshly resected tissue specimens of breast carcinoma tissue and normal tissue were fixed with 10% formalin and embedded in paraffin. The tissue specimens were cut into 4 μm thick serial sections using a microtome and heated at 60°C for eight hours. IHC staining was performed according to the following procedure. Paraffin sections were deparaffinized and hydrated, followed by three washes with phosphate-buffered saline (PBS) for three minutes each. The sections were immersed and rinsed with distilled water before antigen retrieval with EDTA buffer (100°C, 20 minutes). After cooling to room temperature, the sections were washed twice with distilled water and twice with PBS for three minutes each. A peroxidase blocking solution was added dropwise, and the sections were incubated at room temperature for 10 minutes to block endogenous peroxidase activity. After three washes with PBS for three minutes each, non-immune animal serum was added dropwise to the sections, which were then incubated at room temperature for 10 minutes. Then, the residual serum was decanted, and an anti-NIK primary antibody was added dropwise to the sections (Santa Cruz, USA; 1:50 dilution). The specimens were incubated at room temperature for 60 minutes, and PBS was used as a negative control. Three washes were performed with PBS for three minutes each. Then, a biotinylated secondary antibody was added dropwise to the sections (Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China; goat anti-rabbit IgG), which were then incubated at room temperature for 10 minutes. After three washes with PBS for three minutes each, 5 μL of streptavidin-peroxidase was added to the sections, which were then incubated at room temperature for 30 minutes. Three washes were performed with PBS for three minutes each, and a color reaction was performed with a DAB chromogenic reagent. Furthermore, the sections were washed with tap water, counter-stained with hematoxylin, and rinsed with tap water. Next, the sections were dehydrated with alcohol, cleared with xylene, and mounted with neutral gum. Hematoxylin and eosin (HE) staining was performed following standard procedures.

The results of IHC staining were determined as previously reported [5]. Three experienced pathologists independently interpreted the results using a semi-quantitative scoring method. For each section, cells were counted in 15 fields of view at high magnification (400×) using a double blinded method. The percentage of positive cells was calculated as the average number of NIK-positive cells for every 100 cells counted. IHC staining was considered positive upon the emergence of brownish-yellow grains in the cytoplasm. The scoring criteria were as follows: negative, 0 points; pale yellow, 1 point; yellow, 2 points; and brownish yellow, 3 points. The final scores were interpreted as follows: 0-2 points, negative and 3-7 points, positive. The percentage of positive cells was interpreted as follows: 0%-5%, negative; 6%-25%, weakly positive; 26%-75%, moderately positive; and ≥ 76%, strongly positive. Weakly, moderately, and strongly positive were defined as positive results.

Statistical analysis

Data were statistically analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Count data were analyzed with a χ^2 test. A survival analysis was performed using the Kaplan-Meier meth-

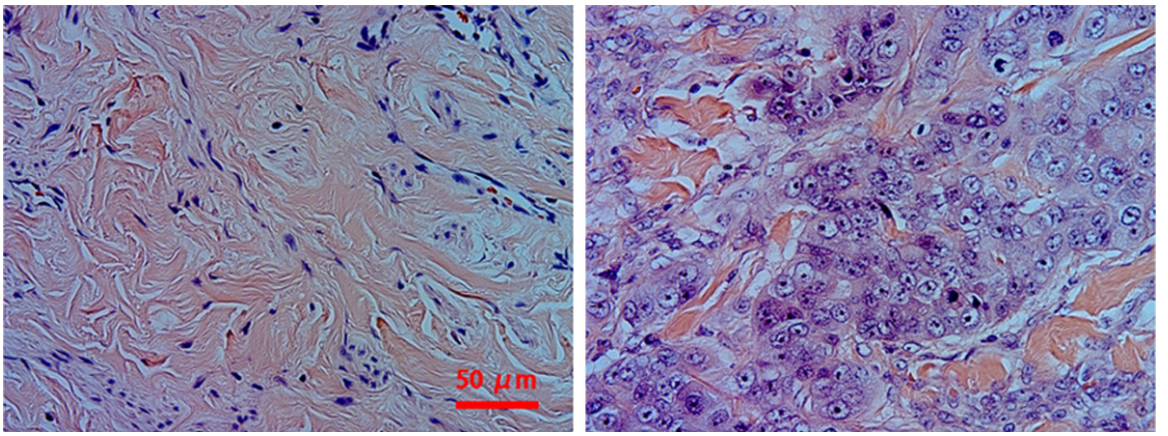

Figure 1. HE staining of breast carcinoma and normal tissue (left: normal tissue; right: breast carcinoma tissue).

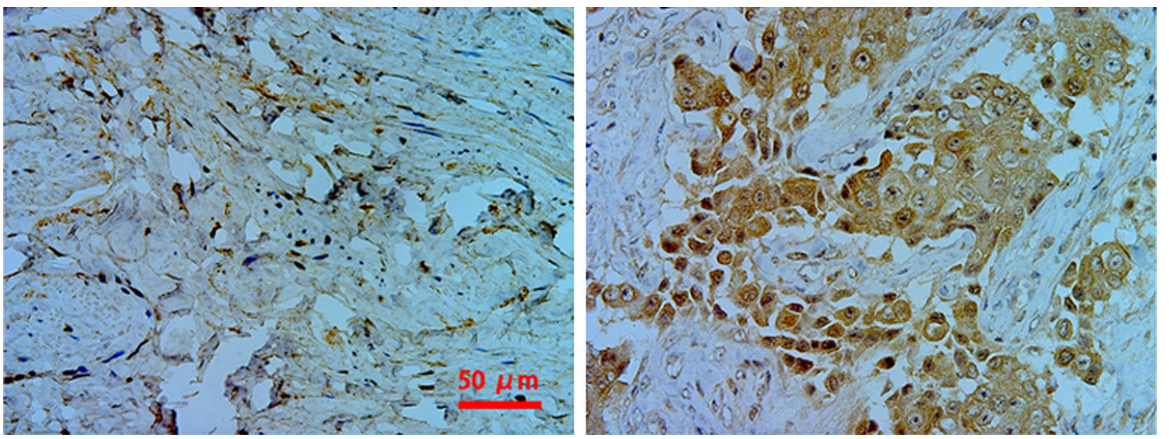

Figure 2. Immunohistochemical staining of NIK in breast carcinoma and normal tissue (left: normal tissue; right: breast carcinoma tissue).

Table 1. Expression of NF-κB-inducing kinase (NIK) in breast carcinoma and tumor-adjacent normal breast tissue

Group	Total cases	NIK		P
		Positive cases (%)	Negative cases (%)	
Breast carcinoma	82	53 (63.4)	29 (36.6)	< 0.001
Normal tissue	82	21 (25.6)	61 (73.4)	

od. A *P* value of less than 0.05 was considered to indicate a significant difference.

Results

NIK protein expression in breast carcinoma tissue and its significance

Figure 1 presents the results of HE staining of typical breast carcinoma tissue and normal tissue. HE and IHC staining showed positive results of NIK staining for the cytoplasm, with pale yellow, brownish-yellow, and brown colors. The experimental results revealed the presence of NIK expression in both breast carcinoma and normal tissue. The number of NIK-positive cells in the breast carcinoma tissue was significantly higher than that in the normal tissue (**Figure 2**). The statistical results showed that the positive rate of NIK expression in breast carcinoma tissue was 63.4%, and that of NIK expression in normal tissue was 25.6%, indicating a significant difference between the two groups (**Table 1**, P < 0.05).

Relationship between NIK protein expression and clinicopathological features of breast carcinoma patients

After demonstrating the increased expression of NIK in breast carcinoma tissue, the association between NIK expression and the clinico-

Table 2. Association between NF-κB-inducing kinase (NIK) expression and the clinicopathological features of 82 patients with breast carcinoma

Group	Cases	NIK		P
		Positive cases	Negative cases	
Age (years)				
< 50	36	22	14	0.349
≥ 50	46	31	15	
Tumor size				
< 3 cm	49	31	18	0.752
≥ 3 cm	33	22	11	
Pathological type				
Ductal carcinoma	40	27	13	0.597
Lobular carcinoma	25	14	11	
Medullary carcinoma	11	7	4	
Mixed cancer	6	5	1	
TNM staging				
Stage I	21	8	13	0.008
Stage II	31	21	10	
Stage III	30	24	6	
Degree of Differentiation				
High	19	12	7	0.602
Moderate	36	22	14	
Low	27	19	8	
Lymph node metastasis				
Yes	36	32	4	0.003
No	46	21	15	

pathological features of breast carcinoma patients was further analyzed. The results showed that the positive expression rate of NIK was significantly associated with the age, tumor size, and pathological score of patients with breast carcinoma ($P < 0.05$, **Table 2**).

Association between NIK protein expression and the clinical prognosis of patients with breast carcinoma

A Kaplan-Meier survival analysis showed that the five-year survival rate of NIK positive patients was 52.9%, with a median survival time of 35 months. The five-year survival rate of NIK negative patients was 79.3%, with a median survival time of 51.5 months. A significant difference was observed between the two groups ($P = 0.021$, **Figure 3**).

Discussion

Extensive studies have shown that the NF-κB signaling pathway is closely related to the development, progression, and invasion of tumors, and targeted regulation of the NF-κB signaling pathway can modulate these processes in various tumors [6]. The NF-κB family members mainly include p65, p50, p52, c-Rel, and RelB. These factors bind to the IκBα inhibitory subunit of NF-κB in the resting state. However, they must dissociate from IκBα to enter the nucleus from the cytoplasm and further bind to the promoter region of a specific gene to initiate transcription and induce a biological effect. The activation of IκBα is primarily regulated by IκB kinase (IKKα) and NIKβ; the upstream activated tumor necrosis factor (TNF) receptor death protein binds to TNF receptor-associated factor 2 to further activate NIK. Next, the activated NIK selectively activates serl76 of IKKα in the IKK kinase complex, thereby activating IKKα, resulting in the ubiquitination and degradation of IκBα [7]. Therefore, NIK plays a key role in the transduction of the NF-κB signaling pathway.

NF-κB expression has been found to be significantly increased in breast carcinoma tissue compared to tumor-adjacent normal breast tissue. Additionally, NF-κB expression has been found to be closely associated with the pathological grade and clinical stage of patients; the five-year survival rate is significantly lower in patients with increased expression of NF-κB compared to those with low expression of NF-κB [8]. In vitro experiments have shown that the use of a selective inhibitor of NF-κB can significantly inhibit proliferation and apoptosis in breast carcinoma cells, suggesting that the NF-κB signaling pathway may serve as a therapeutic target for the treatment of breast carcinoma [9, 10]. With regard to the role of NIK in the activation of NF-κB, increased NIK expression has been implicated in various tumor tissues such as prostate cancer, glioma, and melanoma [11]. Inhibition of NIK expression can markedly inhibit proliferation and apoptosis in tumor cells [12], suggesting that NIK may serve as a potential target for cancer therapy [13]. The present study found that the expression level of NIK was significantly increased in breast carcinoma tissue compared to normal tissue.

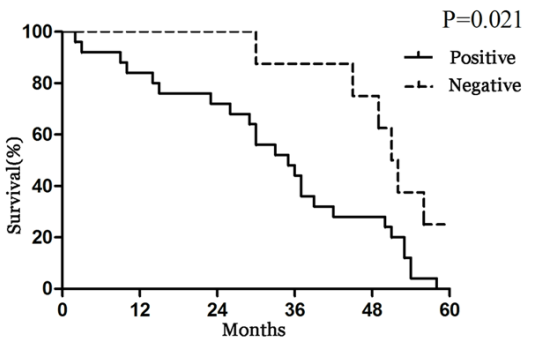

Figure 3. The survival curves of patients with differential expression of NIK were generated according to the Kaplan-Meier method.

This result is highly consistent with previous reports regarding increased expression of NF-κB in breast carcinoma tissue. Further analysis revealed a significant association of NIK with the pathological grade and clinical stage of patients with breast carcinoma; however, no close association was found with patient age or tumor size. Moreover, a Kaplan-Meier survival analysis showed that the five-year survival rate was significantly lower in patients with increased expression of NIK than in those with lower expression of NIK. This result suggests that NIK is an important factor that affects the prognosis of patients with breast carcinoma. In vitro cell culture and animal experiments are needed to further investigate the role of NIK in the development and progression of breast carcinoma. Relevant research will provide new ideas and methods for the clinical treatment of patients with breast carcinoma.

In summary, NIK expression was markedly increased in the tumor tissue of patients with breast carcinoma and was significantly associated with the clinical stage and prognosis of the patients. NIK-targeted therapy may have great implications for the treatment of breast carcinoma. Early detection of NIK expression in breast carcinoma tissue may help to predict the prognosis of breast carcinoma.

Disclosure of conflict of interest

None.

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