EXPRESSION AND SIGNIFICANCE OF ERK PROTEIN IN HUMAN BREAST CARCINOMA

ZHANG Xiu-mei 张秀梅 1, LI Bai-lin 李柏林 2, SONG Min 宋敏 3*, SONG Ji-ye 宋继业 3

1 Department of Pathology, The Third People’s Hospital of Dalian, Dalian 116033; 2 Department of Chemistry, 3 Department of Pathology, College of Basic Medical Science, China Medical University, Shenyang 110001

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ABSTRACT

Objective: To investigate the expression of ERK and p-ERK protein in human breast cancer and their corresponding tissue, to assess the significance of ERK signal pathway in tumorigenesis and progression of breast carcinoma. Methods: 40 breast cancer cases were used in S-P immunohistochemistry technique and Western Blot study. Results: The expression of ERK1, ERK2, and p-ERK protein levels increased remarkably in breast cancer tissues in comparison to normal tissues (P<0.01). The expression was upregulated by 1.32-, 1.53- and 4.27-fold, respectively. The overexpressions of ERK1, ERK2, and p-ERK proteins were obviously correlated with clinical stage of breast cancer. Protein levels of ERK and p-ERK were higher in stage III patients than in stage I and stage II patients (P<0.05). These proteins were strongly related with axillary lymph node metastasis of breast cancer, but not correlated with histopathological type and status of ER and PR of breast cancer. Expression of ERK1, and ERK2, protein showed a positive linear correlation. Conclusion: ERK signal transduction pathway is a key factor during human breast tumorigenesis and breast cancer progression.

Key words: Extracellular signal-related kinase; Breast carcinoma; Phosphorylation; Immunohistochemistry; Metastasis

Breast carcinoma is one of the most common malignancies affecting women. The etiology and pathogenesis of breast carcinoma remain unclear, but several observations suggest a prominent role for the mitogen-activated protein kinase signal pathway in the development of breast carcinoma. Extracellular signal-regulated kinase pathway is one of the important, best characterized and human cancer close related pathway among MAPK regulatory network. ERK is the first discovered member of MAPK family. ERK1 and ERK2 are two major isozymes, which molecule weight of 44- and 42-kiodalton respectively. They are members of the conserved and ubiquitously expressed serine/threonine kinases and can be phosphorylated and activated by a number of growth factors, cytokines, hormones and mitotic signals, and are involved in cell cycle and cell proliferation, differentiation and apoptosis resistance. Our objective is to assess the significance of ERK signal pathway in tumorigenesis and progression of breast carcinoma.

MATERIALS AND METHODS

Specimens

Forty breast cancer cases were used in immunohistochemistry study. These cases were obtained from Shenyang Military Hospital and the First Affiliated Hospital of China Medical University from June 1998 to October 2000. All the specimens were fixed in formalin and embedded with wax. 40 fresh cases with their adjacent normal breast tissues used in Western Blot were obtained from the first Affiliated Hospital of China Medical University from October 2002 to March 2003. These specimens were divided into two parts: one frozen immediately at -7°C and the other fixed with formalin and embedded into paraffin block. None of the patients received chemical
or radioactivity therapy.

Reagents

Rabbit polyclonal antibody against ERK1 (sc-94), ERK2 (sc-154) and mouse monoclonal antibody against p-ERK (sc-7383) were purchased from Santa Cruz Biotechnology Company. Alkaline phosphatase conjugated goat anti-rabbit antibody was purchased from Beijing Zhongshan Biotechnology Company. S-P reagent kit (KIT-9710) and DAB agent kit (DAB-0030) were purchased from Fuzhou Maxim Company. Alkaline phosphatase conjugated goat anti-mouse antibody and NBT/BCIP KIT were purchased from Huamei Company.

Methods

Western Blot

The tissues (about 3 mm³) were homogenized in lysis buffer, then centrifuged to pellet insoluble material. Proteins were electrophoretically transferred from gel to PVDF and incubated with primary antibody and enzyme second antibody. The results were scanned and measured by computer image analysis system.

Immunohistochemistry

To detect the expression of ERK, p-ERK in 40 cases of breast cancer by immunohistochemistry S-P method, 500 tumor cells were observed under high magnification per slide at random. ERK1, ERK2 and p-ERK immunostaining was classified according to following: (a) Negative, less than 10% of staining cells. (b) Mild positive, immunostaining was weak or strong staining cells were more than 10% and less than 50%. (c) Strong positive, strong staining cells were more than 50%.

Statistical Analysis

SPSS version 10.0 for Windows was used in the analysis. Chi-squared test was used to clarify the relationship between staining results and other variables studied. Linear correlation of ERK1 and ERK2 was determined by calculating Pearson’s correlation coefficient. Paired T test was used to compare ERK1 and ERK2 and p-ERK protein expression in breast cancer with those in the adjacent non-cancerous lesion of each case. P values less than 0.05 were considered to be statistically significant.

RESULTS

The Expression of ERK1, ERK Proteins in Breast Cancer

The results of Western Blot analysis suggested that the expression of ERK1, ERK2 and p-ERK protein levels increased remarkably in breast cancer tissues in comparison to normal tissues (P<0.01). The expression was upregulated by 1.32-, 1.53- and 4.27-fold, respectively (Table 1, Figure 1-3).

Table 1. The results of Western blot of the expression of ERK1, ERK2 and p-ERK in breast carcinoma and normal breast tissue (x±s)

<table>
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<th>n</th>
<th>The gray value</th>
<th>t</th>
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<td>Breast tumor</td>
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<tr>
<td>ERK2</td>
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<td></td>
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<tr>
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<td>p-ERK</td>
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Fig. 1. ERK1 expression analyzed by Western blot. T: Tumor; N: Normal breast tissues

Fig. 2. ERK2 expression analyzed by Western blot. T: Tumor; N: Normal breast tissues