EXPRESSION OF SURVIVIN AND E-CADHERIN IN BREAST CANCER

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ABSTRACT

Objective: Survivin is a member of the inhibitor of apoptosis (IAP) family, and is involved in the regulation of cell division. Survivin expression is related with poor survival of patients with tumors including neuroblastoma, non-small-cell lung cancer, breast carcinoma, gastric carcinoma, rectal cancer, recurrent colorectal carcinoma and bladder cancer. It is thought to be an important prognostic marker in cancers[1,2]. E-cadherin functionally belongs to transmembrane glycoproteins family, it is responsible for intercellular junction mechanism that is crucial for the mutual association of vertebrate cells. Several studies indicated that in carcinomas E-cadherin functions as an invasion suppressor molecule such that its loss permits or enhances the invasion of adjacent normal tissues[3]. In this study, we investigated the relationship between the expression of surviving gene, E-cadherin gene and invasion clinicopathological features of breast cancer. Methods: The expression of surviving gene and E-cadherin were detected by SP immunohistochemical technique in tissues of 66 breast cancer, 20 breast fibroadenoma and 20 adjacent breast tissue. Results: The positive rate of surviving gene expression in breast cancer was 42.2%, significantly higher ($P=0.025$) than those in breast fibroadenoma (35.0%), and adjacent breast tissue (10.0%). The positive rate of E-cadherin in the groups of adjacent breast tissue, breast fibroadenoma and breast cancer were 100%, 100% and 42.4%, there was significant difference between the group of benign and malignant tumor ($P=0.005$). The positive rate of surviving in breast cancer with local lymph node metastasis was significant higher than that in breast cancer without lymph node metastasis ($P=0.01$), and E-cadherin in breast cancer with local lymph node metastasis was significant lower than that without lymph node metastasis ($P=0.01$). There was no significant difference among the groups of pathological types and TNM stages in the expression of surviving ($P=0.966$ & $P=0.856$), but there was significant difference in the expression of E-cadherin among these groups ($P=0.01$ & $P=0.023$). Conclusion: The loss or decrease of E-cadherin expression may promote the exfoliation of cancerous cells from original tissues, and surviving gene may promote the viability of the exfoliated cancer cells and the formation of new metastasis focus. These 2 factors cooperate with each other in the process of metastasis and invasion. They have a close relation with the breast cancer clinicopathological features.

Key words: Breast cancer; E-cadherin; Survivin gene
MATERIALS AND METHODS

Patient Selection

Specimens were taken from 66 cases of breast cancer, 20 cases of breast fibroadenoma, and 20 cases of adjacent normal tissue. All patients were women, and underwent operation at the Second Affiliated Hospital of Dalian Medical University from 2001 to 2002. The age of the patients ranged from 35 to 65 years (average 53.5 years). Of 66 cases of breast cancer, 22 were in TNM stage I, 20 in stage II and 24 in stage III; 30 with axillary lymph node metastasis, and 36 without axillary lymph node metastasis; 26 were invasive lobular carcinoma, and 40 were invasive ductal carcinoma.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue specimens were stained by SP immunohistochemistry technique for surviving and E-cadherin detection. Five-micron sections were dewaxed in xylene, incubated in 3% hydrogen peroxide for 5 minutes to eliminate intrinsic peroxidase, washed in phosphate-buffered saline (PBS) for 3 minutes, bathed in 0.01 mol/L sodium citrate buffer (pH 6.0) after bringing the solution to boil in a pressure cooker, and boiled for 4 minutes while maintaining the pressure. The slides were then washed in PBS after refrigeration, quenched in normal goat serum for 20 minutes, incubated overnight at 4°C with monoclonal antibody raised against purified recombinant E-cadherin (4A2 C7) or surviving (4F7). Then antimouse immunoglobulin and streptavidin conjugated to horseradish peroxides were added. Finally, 3,3'-diaminobenzidine was used for color development, and hematoxylin was used for counter staining. E-cadherin (4A2 C7), surviving (4F7) antibody and antimouse immunoglobulin were purchased from Beijing Zhongshan Golden Biotechnology Co., Ltd., Beijing.

Quantification of Immunostaining

E-cadherin was mainly stained on membrane in brown, partly stained in cytoplasm. Survivin was stained in cytoplasm and/or nucleus. Their immunoreactivity was evaluated semi-quantitatively according to the percentage of positive cells. Positive cells were assessed in at least 5 high power fields at 400 multiple signal magnification. For E-cadherin, when positive cell were (a) 0% of tumor cell, negative (-); (b) <5%, positive (+); (c) 5%—75%, positive (++); (d)>75%, positive (+++)46. Survivin positive cells were assigned an arbitrary scores as follows; 0<5%; 1=6%—25%; 2=26%—50%; 3=51%—75%, 4=76%—100%5]. For practical and statistical purposes, subgroup (a), (b) and (c) or score 0 and 1 were considered as negative, and (d) or score 2, 3, and 4 were defined as positive.

Statistical Analysis

All statistical analyses were performed using the SPSS 10.0 software, correlation between antigen expression and other clinicopathological parameters was studied by the X^2 statistic, a value of P<0.05 was considered to indicate significance.

RESULTS

Breast cancer cells showed strong surviving brown immunostaining in nucleus and cytoplasm of the 66 breast cancer specimens examined, the positive rates of surviving in adjacent breast tissue, fibroadenoma, and breast cancer were 10.0% (2/20), 35.0% (7/20), and 42.4% (28/66) respectively, there was significant difference between the groups of benign and malignant tumor (P<0.025) (Table 1). The positive rates of surviving in breast cancers of TNM stage I, II and III were 27.2% (2/22), 30.0% (6/22), and 66.7% (16/24) respectively, there was no significant difference among the groups (P=0.856). The positive rates of surviving in group with local lymph node metastasis (66.7%, 20/30) was significantly higher than that without lymph node metastasis (22.2%, 8/36) (P<0.01). There was no significant difference between invasive ductal (40.0%, 16/40) and lobular cancer (40.2%, 12/26) (P=0.966).

E-cadherin expression in breast cancer cells was mainly detected on the cell membrane. The positive rate of E-cadherin in normal breast tissue, fibroadenoma, and breast cancer were 100% (20/20), 100% (20/20), and 42.4% (38/66) respectively, there was significant difference between the groups of benign and malignant tumor (P<0.005) (Table 2). The positive rates of E-cadherin in breast cancer of TNM stage I, II and III were 72.7% (16/22), 40.0% (8/20), and 16.7% (4/24) respectively, there was a significant difference among the groups with different stages (P<0.023). The positive rates of E-cadherin in breast cancer of TNM stage I, II and III were 72.7% (16/22), 40.0% (8/20), and 16.7% (4/24) respectively, there was a significant difference among the groups with different stages (P<0.023). The positive rates of E-cadherin in breast cancer of TNM stage I, II and III were 72.7% (16/22), 40.0% (8/20), and 16.7% (4/24) respectively, there was a significant difference among the groups with different stages (P<0.023).

DISCUSSION

The process of cancer invasion and metastasis consists of a complex series of sequential steps and depends on the properties of specific tumor cells and