EJGO European Journal of Gynaecological Oncology

# Significance and expression of c-erBb-2, p53, and caspase-3 in breast cancer tissue in different age groups

**Z.Y. Liu[1], L. Guo[1], G. Xiao[1], G.Y. Dong[2], Y.X. Zhang[2], H. Cheng[2], X.Y. Wang[2], C. Yang[1]**

*[1]Department of Breast Surgery, [2]Department of Pathology, Tangshan Maternity and Child Health Care Hospital, Tangshan (China)*

**Summary**

*Objective:* The aim of this study was to explore the expression of c-erBb-2, p53, and caspase-3 in breast cancer tissue in different age groups. *Materials and Methods:* One hundred seventy-two patients with breast cancer were divided into two groups by age (≤ 35 and ≥ 40 years). Immunohistochemical analysis was performed to detect the expression of c-erBb-2, p53, and caspase-3 in the tumor tissue from the patients. The authors compared the expression levels between the two groups. *Results:* In 90 young women with breast cancer, the positive expression rate of c-erBb-2, p53, and caspase-3 was 41.1% (37/90), 67.8% (61/90), and 35.6% (32/90), respectively, and it was 23.2% (19/82), 40.8% (39/82), and 57.3% (47/82), respectively, in the older patients. In the young group, the expression of caspase-3 was significantly lower ($p < 0.05$) and the expression of c-erbB-2 and p53 were significantly higher ($p < 0.01$). *Conclusion:* The biological behavior of breast cancer and apoptosis is associated with the expression of p53 and c-erbB-2; thus, more attention should be paid to the expression of cell apoptotic factors when treating and assessing the prognosis of patients with breast cancer.

*Key words:* Breast cancer; Apoptotic factor; Immunohistochemical assay; Prognosis.

## Introduction

Breast cancer is one of the most common malignant tumors and has become the most common cause of cancer deaths among women worldwide [1, 2]. In recent years, the incidence of breast cancer has increased in China [3]. It has become more important to recognize breast cancer and take effective measures to treat it, which can improve its prognosis and patients' quality of life. Nowadays, many experts believe that the development of tumors is associated with aberrant cell apoptosis [4-6]. Many researchers have studied the relationships among the expression of estrogen receptors (ER), progesterone receptors (PR), HER2/neu, and breast cancer and tumor pathological characteristics [7-10]. However, there are fewer reports about the relationships among oncogenes, tumor suppressor genes, and apoptotic factors. This study investigated the expression of c-erBb-2, p53, and caspase-3 in breast cancer tissues in patients of different ages, and explored the possible mechanism of breast cancer biological behavior related to cell apoptosis to provide information useful for the prognosis and treatment of breast cancer.

## Materials and Methods

One hundred seventy-two breast cancer specimens were collected from January 2012 to December 2014. The breast cancers were confirmed by pathology. All of the patients were women, and 90 patients were classified as the young group, 23- to 35-years-old (median age: 30.5 years), and 82 patients were in the older group, 40- to 73-years-old (median age: 54.1 years). None of the patients in either group received any chemotherapy. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Tangshan Maternity and Child Health Care Hospital. Written informed consent was also obtained from all participants.

Mouse anti-human monoclonal antibodies for c-erBb-2, p53, and caspase-3 were obtained. Avidin-biotin peroxidase immunohistochemistry was used in this study to detect expression of the proteins. A known positive specimen and phosphate-buffered saline (PBS) were the positive and negative controls, respectively. All positive reactions showed a brown color. The p53 positive staining was mostly observed in the nucleus, with some staining in the cytoplasm. c-erbB-2 positive staining was observed in the cell membrane and cytoplasm, and positive staining for caspase-3 was observed in the cytoplasm or nucleus.

Statistical analysis was performed using SPSS 13.0 software. Chi-square tests were used to compare the two groups, with $p > 0.05$ denoting no significant statistical difference. Spearman rank correlation was used to analyze the correlation among the clinical pathological parameters.

## Results

As shown in Table 1, in the 90 breast cancers from young patients, the positive rate of c-erBb-2, p53, and caspase-3 expression was 41.1% (37/90), 67.8% (61/90), and 35.6% (32/90), respectively, and in the older breast cancer group, the positive rate of c-erBb-2, p53, and caspase-3 expression was 23.2% (19/82), 40.8% (39/82), and 57.3% (47/82), respectively. In the younger group, the expression level of caspase-3 was lower ($p < 0.05$) and those of c-erbB-2 and

Revised manuscript accepted for publication November 16, 2016

Table 1. — *Expression of cerBb-2, p53 and caspase-3 in two groups.*

| Group | CerbB-2 | | | p53 | | | Caspase-3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | + | – | Rate (%) | + | – | Rate (%) | + | – | Rate (%) |
| Young | 37* | 53 | 41.1 | 61* | 29 | 67.8 | 32▲ | 58 | 35.6 |
| Elderly | 19 | 63 | 23.2 | 39 | 41 | 40.8 | 47 | 35 | 57.3 |

*$p < 0.01$ compared with the elderly breast cancer group; ▲ $p < 0.05$ compared with the elderly breast cancer group.

p53 were higher ($p < 0.01$), with a statistically significant difference between groups for all three proteins.

## Discussion

Recent studies indicate that the occurrence of breast cancer is associated not only with activation of oncogenes and mutation or deletion of tumor suppressor genes, but also with inhibition of tumor cell apoptosis [11, 12]. With a decrease in tumor cell apoptosis, tumor cells grow fast and their ability to invade is enhanced.

c-erbB-2 is an oncogene that is activated by some endogenous and exogenous factors. Gene amplification or high expression of c-erbB-2 results in tumor transforming activity [13]. p53 mutations are common in malignant tumors. Mutations in the p53 gene prevent it from performing its function of surveillance and inducing apoptosis. Cells with p53 mutations accumulate gene mutations and chromosome aberrations because of unstable inheritance [1], which causes cell transformation and cancer [14-16]. In the present study, expression of c-erbB-2 and p53 was significantly higher in breast cancers from younger women, which further confirms the relationship between c-erbB-2 and p53 [2]. Furthermore, high expression of c-erbB-2 and p53 indicate a breast cancer with certain characteristics, such as strong invasion ability, high malignancy, and a poor prognosis for the patient [17, 18].

The caspase family plays an important role in the process of cell apoptosis [19]. Caspase-3, located downstream in signaling cascades, is an apoptotic effector. The inhibition of activation of caspases causes a disorder of cell apoptosis, which disrupts the balance between cell apoptosis and cell proliferation, allowing cancers to form [20]. Some studies found that downregulation of caspase-3 genes is closely correlated with potential transformation, recurrence, and poor prognosis [21]. Meanwhile, another study reported that the positive rate of caspase-3 protein expression was lower in breast cancer than benign breast tissue, and the lower the positive rate of caspase-3 protein, the higher the clinical stage [22].

The present study found that the expression of caspase-3 was lower in breast cancers from younger as compared to older women, which demonstrates that in young women, cell apoptosis was significantly inhibited during development of breast cancer, which resulted in malignancy and a poor prognosis.

This study also investigated the expression of p53, and the results for p53 were opposite to that of caspase-3. One possible reason is that p53 is involved in the regulation of caspase-3. Some studies indicate that p53 depends on the activation of caspase-3 and the apoptotic mechanism of p53 involves a p53-caspase-4 complex inhibiting the catalytic activity of caspase-3 [23].

The present authors conclude that high expression of p53 promotes the high expression of c-erBb-2 and inhibits the activity of caspase-3, which indicates that cell apoptosis is significantly inhibited in tumors from younger women as compared to older women, and the growth of the tumor is therefore out of control, resulting in the higher malignancy of breast cancer in younger women.

In summary, the biological properties of breast cancer are not only correlated to the different expression levels of p53 and c-erbB-2, but also with the inhibition of cell apoptosis. Therefore, we should focus on the expression of cell apoptotic factors in the treatment of patients with breast cancer, which will help us to exactly assess their prognosis and provide insight into directions for developing therapies for breast cancer.

## References

[1] Homan S.G., Yun S., Stewart B.R., Armer J.M.: "Breast Cancer Survivorship Care: Targeting a Colorectal Cancer Education Intervention". *J. Pers. Med*., 2015, *5*, 296.
[2] Kerr J.F., Winterford C.M., Harmon B.V.: "Apoptosis. Its significance in cancer and cancer therapy". *Cancer*, 1994, *73*, 2013.
[3] Stewart B.W., Kleihues P.: "World Cancer Report". Lyon: IARC Press, 2003, 188.
[4] Evan G., Littlewood T.: "A matter of life and cell death". *Science*, 1998, *281*, 1317.
[5] Marx J.: "Cell death studies yield cancer lcues". *Science*, 1993, *259*, 760.
[6] Kerr J.F., Winterford C.M., Harmon B.V.: "Apoptosis. Its significance in cancer and cancer therapy". *Cancer*, 1994, *73*, 2013.
[7] Lower E.E., Glass E.L., Bradley D.A., Blau R., Heffelfinger S.: "Impact of metastaticestrogen receptor and progesterone receptor status on survival". *Breast Cancer Res. Treat*., 2005, *90*, 65.
[8] Hayes D.F., Thor A.D.: "c-erbB-2 in breast cancer: development of a clinicallyuseful marker". *Semin Oncol*., 2002, *29*, 231.
[9] Li F.Y., Wu S.G., Zhou J., Sun J.Y., Lin Q., Lin H.X., *et al*.: "Prognostic value of ki-67 in breast cancer patients with positive axillary lymph nodes: a retrospective cohort study". *PLoS One*, 2014, *9*, e87264.
[10] Guarneri V., Generali D.G., Frassoldati A., Artioli F., Boni C., Cavanna L., *et al*.: "Doubleblind, placebo-controlled, multicenter, randomized, phase IIbneoadjuvant study of letrozole-lapatibib in postmenopausal hormone receptor-positive, human epidermal growth factor receptor 2-negative, operable breast cancer". *J. Clin.*

*Oncol*., 2014, *32*, 1050.
[11] Xiao Z., Shan J., Li C., Luo L., Lu J., Li S., *et al*.: "Mechanisms cyclosporine-induced renal cell apoptosis: a systematic review". *Am. J. Nephrol*., 2013, *37*, 30.
[12] Xu C., Wu A., Zhu H., Fang H., Xu L., Ye J., *et al*.: "Melation is involved in the apoptosis and necrosis of pancreatic cancer cell line SW-1990 via modulating of Blc-2/Bax balance". *Biomed. Pharmacother*., 2013, *67*, 133.
[13] Sun L., Liu J., Wang S., Chen Y., Li Z.: "Prevalence of BRCA1 gene mutation in breast cancer patients in Guangxi, China". *Int. J. Clin. Pathol*., 2014, *7*, 6262.
[14] Achari C., Winslow S., Larsson C.: "Down Regulation of CLDND1 Induces Apoptosis in Breast Cancer Cells". *PLoS One*, 2015, *10*, e0130300.
[15] Walerych D., Napoli M., Collavin L., Del Sal G.: "The rebel angel: mutant p53 as the driving oncogene in breast cancer". *Carcinogenesis*, 2012, *33*, 2007.
[16] Muller P.A., Vousden K.H., Norman J.C.: "P53 and its mutants in tumor cell migration and invasion". *J. Cell. Biol*., 2011, *192*, 209.
[17] Brzdar A., Jonat W., Howell A., Jones S.E., Blomqvist C., Vogel C.L., *et al*.: "Anastmzole, a potent and selective aromatase inhibitor, versusmegestrol acetate in postmenopausal women with advanced breast cancer: resultsof overview analysis of two phase III trials. Arimidex study group". *J. Clin. Oncol*., 1996, *14*, 2000.
[18] Mesri M., Morales-Ruiz M., Ackermann E.J., Bennett C.F., Pober J.S., Sessa W.C., *et al*.: "Suppression of vascular endothelial growth factor-mediated endothelial cell protection by surviving targeting". *Am. J Pathol*., 2001, *158*, 1757.
[19] Huang Q., Li F., Liu X., Li W., Shi W., Liu F.F., *et al*.: "Caspase3-mediated stimulation of tumor cell repopulation during cancer radiotherapy". *Nat. Med*., 2011, *17*, 860.
[20] Liu B., Han M., Wen J.K., Wang L.: "Livin/ML-IAP as a new target for cancer treatment". *Cancer Lett*., 2007, *250*, 168
[21] Golubovskaya V., Beviglia L., Xu L.H., Earp H.S. 3rd, Craven R., Cance W.: "Dual inhibition of focal adhesion kinase and epidemal growth factor receptor pathwayscooperatively induces death receptor-mediated apoptosis in human breast cancer cells". *J. Biol. Chem*., 2002, *277*, 38978.
[22] Krajewaka M., Wang H.G., Krajewaki S., Zapata J.M., Shabaik A., Gascoyne R., *et al*.: "Immunohistochenmicalanalysis of in vivo patterns of expression of CPP32(Caspase-3), a cell death proteinase". *Cancer Res*., 1997, *57*, 1605.
[23] Aleo E., Henderson C.J., Fontanini A., Solazzo B., Brancolini C.: "Identification of new compounds that trigger apoptosome-independent caspase activation and apoptosis". *Cancer Res*., 2006, *66*, 9235.

Corresponding Author:
XIAO GANG, M.D.
Department of Breast Surgery
Tangshan Maternity and Child Health Care Hospital
14 South Jianshe Road
Tangshan 063000 (China)
e-mail: gangxiaodoc@163.com